Quantitative Assessment of the outcome of Anti-Vascular Endothelial Growth Factor Treatment for Neovascular Macular Degeneration

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Abstract

Neovascular Macular Degeneration is a significant cause of blindness world–wide. Anti-Vascular Endothelial Growth Factor medication injected directly into the eye has halved the disease burden in recent years.

Being able to adequately quantify the outcome of neovascular AMD treatment gives perspective not only on appropriate intervention for individual patients, but also the understanding of the science behind the disease, its therapeutics and design of future clinical trials.

Assessment tools can be either subjective or objective, and often interrogate either efficacy or safety endpoints. Although patient reported Quality of Life utilities give the ultimate assessment of treatment success for an individual, surrogate biomarkers are more effective in judging short-term response. Visual acuity assessment is useful in analyzing cohorts of individuals, but its subjective nature means that it is not particularly useful in determining individual retreatment decisions. An objective morphological assessment of the macular architecture does provide a good way of assessing short-term response however. Retinal sensitivity also demonstrates usefulness as an endpoint for clinical trials, but as of yet is too cumbersome a technique for high volume clinical work. Functional imaging of the visual cortex remains a research tool at present, but provides promise as a new objective endpoint. Importantly this thesis has confirmed that that cortex is able to regain function after a short period of compromise due to neovascular macular degeneration.

Measurement tools to assess the outcome of treatment are best selected, often in composite, with regards to the prime reason for assessment being undertaken.

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Acknowledgments

Author's Declaration

The following statement clarifies the nature and extent of contribution to the research by colleagues. Otherwise the work is my own and has not been submitted previously for any other degree at this or any other university.

Chapter 2.

Data collection was assisted by Dr. Patrizia Tschour, Dr. Divya Venugopal and statistical evaluation verified by Dr. Victoria Allgar. These data formed a manuscript, which is now in print (Tschour P., Pilly B., Venogopal D., Gale R. (2013). Optimising assessment intervals improves visual outcomes in ranibizumab treated age-related neovascular macular degeneration: using the stability phase as benchmark. Graefes Arch Clin Exp Ophth, 251(10), 2327-30.).

Chapter 3.

I was the Principal Investigator with site level responsibility for the study. Data collection was assisted by Dr. Patrizia Tschour. These data contributed to a national data set, which resulted in two publications (Tufail A., Narendran N., Patel P., Sivaprasad S., Amoaku W, Gale R., et al. (2013b) Ranibizumab in myopic choroidal neovascularization: the 12-month results from the REPAIR study. Ophthalmology, 120, 1944-1945 e1941; Tufail A., Patel P., Sivaprasad S., Amoaku., Browning A. Gale R., et al. (2013a). Ranibizumab for the treatment of choroidal neovascularisation secondary to pathological myopia: interim analysis of the REPAIR study. Eye, 27, 709-715.).

Chapter 4.

I was the principal Investigator with site level responsibilities. Data collection was assisted by Dr. Patrizia Tschour. The data contributed to a

national dataset, which is now published (Amoaku W., Gale R., Lotery A., Geeta M., Sivaprasad S., Petrillo J., et al. (2015). Treatment Satisfaction and Well-being in Patients With Myopic Choroidal Neovascularization Treated With Ranibizumab in the REPAIR Study. Plos one, accepted for publication.).

Chapter 5.

Prof. Antony Morland was the chief investigator for this study. Data collection was assisted by Dr. Patrizia Tschour, Dr. Archana Airody and Debbie Wood. Statistical analysis was assisted by Dr. Alexandra Mankowska. Data has been presented at The Association for Research in Vision and Ophthalmology congress 2015 (Mankowska A., Airody A., Baseler H., Morland A., Gale R. (2015). Analysis of an area of interest (choroidal neovascular membrane and sequelae) using microperimetry demonstrates significant increase in retinal sensitivity following anti-VEGF therapy for neovascular Age-related Macular Degeneration (nvAMD). ARVO. Program no 2772, Poster no B0154.)

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Chapter 7.

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Preface

Neovascular or 'wet' macular degeneration is a devastating condition, which left untreated leads to irreversible and severe central visual loss. Although typically presenting in one eye it affects the fellow eye in around half of the time within 5 years. Thankfully injectable treatments into the eye that target the key chemical driver of the disease are available, and these often give partial visual recovery with long-term stability. Blind registration has reduced by half.

Being able to quantify the outcome of treatment, both in terms of safety and efficacy, are paramount. There are many ways of being able to assess treatment outcomes, the principle way being measuring of visual acuity. But is visual acuity a good representation of visual function? Ideally an outcome measure should be objective, reproducible, easy to acquire and relevant to those affected. A greater understanding of outcome tools will allow the clinical and scientific community to appropriately select a technique to assess current and future treatments.

Through a series of 6 studies, each asking questions about the efficacy or safety of treatment of neovascular macular degeneration, different outcome measures are explored.

Chapter 1: An Introduction to Macular Degeneration and the Assessment of Treatment of Neovascular Disease.

1. Overview

Treatments for the blinding disease 'Wet' Macular Degeneration took a significant step forward a decade ago with the introduction of a new class of ocular therapy, Vascular Endothelial Growth Factor Inhibitors. Being able to measure the response to treatment accurately not only increases our understanding of the science behind such treatments but also helps establish suitable treatment endpoints.

After a brief introduction to essential ocular anatomy and the visual pathway this chapter details Macular Degeneration, its subtypes, diagnosis and treatments, all of which are topics essential to the understanding of this thesis. Furthermore, this chapter discusses the commonly used outcome measures of treatments and a selection of research tools, before formulating the aims of this thesis.

1.1 The Macula and vision

Essential aspects of both macro and microscopic anatomy core to the understanding of this thesis are detailed here.

1.1.1 Macroscopic ocular anatomy

The basic concept of the anatomy of the eye is similar to a camera with light rays being refracted at the optical surfaces of the eye, primarily the lens and cornea anteriorly, being focused on the light sensitive tissue, the retina, posteriorly. Between the lens and the retina lies the transparent vitreous gel, which is attached to the retina at the pars plana, 3-4 mm posterior to the cornea.

1.1.2 Microscopic retinal anatomy

Principally three types of cell form the commencement of the visual pathways within the retina; the photoreceptors in the outermost aspect of the neurosensory retina, which synapse with the bipolar cells, which in turn synapse with the retinal ganglion cells at the innermost retina (Snell & Lemp, 1998). In addition, horizontal and amacrine cells provide lateral connectivity between cells within layers, and Muller cells provide glial support across layers.

Light energy is transduced into electrical signals by the retinal photoreceptors in the photo-transduction cycle. The photoreceptors are of two types: rods which are located principally in the peripheral part of the retina and responsible for non-colour, peripheral and low light vision, and cones which are located centrally and are responsible for vision in high illumination settings allowing high acuity. There are three types of cones which have maximal sensitivity in the short, medium and long parts of the visible electromagnetic spectrum often referred to as the blue, green and red cones. It is these cells that are responsible for colour perception at a retinal level. Horizontal cells work 'laterally' in the retina providing support in the form of inhibition and immunomodulation between the photoreceptors and bipolar cells. Amacrine cells play a similar supporting role within the retina acting between the bipolar and retinal ganglion cells (Snell & Lemp, 1998).

There are a number of cells that support the neurosensory aspect of the retina. The Retinal Pigment Epithelium (RPE) lies external to the photoreceptor layer and has a number of functions including supporting the photo-transduction cycle, light absorption, phagocytosis (engulfing foreign material) and ion buffering,. Beneath the RPE is a vascular layer, the choriocapillaris. The choriocapillaris supplies the oxygen and nutrient requirement to the outer layers of the retina. Its innermost layer, the basement membrane termed Bruch's membrane, is shared with the

outermost layer of the RPE and serves as a physical barrier between the vascular system and the retina (Snell & Lemp, 1998).

The macula is the central part of the retina, usually defined as the area located with the principle retinal vascular anatomy and is often mentioned in disease terminology. It is the central part of the macula, the fovea, that has the very highest density of cone photoreceptors and is responsible for our finest visual acuity enabling tasks such as reading (Snell & Lemp, 1998).

1.1.3 The visual pathway

Following their synapse with the bipolar cells, the ganglion cells, of which there are approximately 1 million per eye at birth, form the nerve fibre layer on the inner most aspect of the retina. The axons of the ganglion cells form the optic nerve, which passes posteriorly through the orbit. At the optic chiasm the nasally located fibres decussate and with the temporal fibres of the fellow eye form the optic tracts, which then synapse in the lateral geniculate nuclei (LGN). The onward nerve fibres from the LGN form the optic radiations that ultimately pass into the visual cortex in the posterior aspect of the occipital lobe of the cerebral tissue (Snell & Lemp, 1998).

1.1.4 The visual cortex

The visual cortex is the aspect of the cerebral tissue that is responsible for processing the input from the retina to establish visual perception. The primary visual cortex (which is also called V1 or the striate cortex) is the region of the visual cortex that first receives information from the LGN. A cortical hierarchy of areas termed V2, V3, V4 and V5, collectively known as the extrastriate cortex, then further processes the information. These cortical regions serve different purposes in interpreting the world. For example neurons in V1 and V2 respond selectively to bars of specific orientations and are believed to support edge and corner detection. In addition basic information about color and motion is processed here. (Jessel,

Schwartz and James, 2000). Other areas appear to establish specific functional roles in motion (V5) and colour perception (V4) (Born & Bradley, 2005).

1.2 Macular degeneration

Macular degeneration is a pathological process affecting the central retinal tissues and encompasses a number of well-defined diseases. The term 'degeneration' refers to the 'loss of specialist structure and function' of a tissue, and is a very broad definition that could incorporate most diseases, but is often used when the process is poorly understood and associated with ageing (Underwood, 1992). As knowledge about macular degeneration has grown it transpires that there is a genetic explanation for some of its forms; in the age-related type there are currently 20 known genetic loci responsible for approximately half of disease heritability (Fritsche, et al. 2014). This calls into question the nomenclature 'macular degeneration', and indeed whether in the future it maybe be better suited to being labeled as a form of 'macular dystrophy', a term often reserved for a collection of macular pathologies that have a strong genetic influence (Kanski, 1999). Nonetheless, macular degeneration is a condition with characteristic phenotypes.

1.2.1 Classification of macular degeneration

The commonest form of macular degeneration is Age-related Macular Degeneration (AMD). It is the commonest cause of blindness in the elderly population in the western world, being a condition diagnosed over the age of 50 years (Ferris et al 2013; Klien et al 2007). The second commonest form of macular degeneration is Myopic Macular Degeneration (MMD), often occurring earlier in life. Table 1 illustrates a summary of macular degeneration classification detailed in the following sections.

Macular Degeneration			
A. Age-related Macular Degeneration		B. Non Age-related Macular Degeneration e.g. Myopic, Hereditary, Inflammatory	
1. Dry	2. Wet (or 'neovascular')	1. Dry	2. Wet (or 'neovascular')
a. Early b. Intermediate (non central atrophy) c. Late (central atrophy)	Late (a. Choroidal neovascular membrane, b. Retinal Angiomatous proliferation or c. Polypoidal choroidal, Vasculopathy)	Atrophic	Choroidal neovascular membrane

Table 1. A summary of the classification of macular degeneration.

1.2.1.1 Age-related Macular Degeneration

A common classification of AMD is into 'Dry' and the less common 'wet' forms.

1.2.1.1.1 Dry Age-related Macular Degeneration

As we age a lipid-protein rich byproduct of the photo-transduction cycle, lipofuscin, causes thickening of Bruch's membrane. As a part of the aging process this material accumulates beneath the RPE in bodies called Drusen; they are present in approximately two-thirds of the population over the age

of 70 years (Rickmen et al, 2013). This accumulation is considered to be abnormal or 'degenerative' if there are excessive drusen or additional 'abnormalities' of the RPE. These abnormalities can be seen as hyperpigmentation of the RPE, which is noted as 'stipulation' or larger 'clumping' of pigment, or alternatively there can be loss of the pigmentation termed hypopigmentation. Drusen can resolve or can evolve to become larger or worse still cause loss of the associated photoreceptors and RPE in the process of atrophy (Rickmen et al, 2013). Geographic atrophy (GA) is observed where there is a well-defined area of visible loss of the RPE demonstrated by the clear visualization of the choriocapillaris blood vessels beneath (Sunness et al, 1999).

A well-accepted classification of AMD is that described by the Age-Related Eye Disease Study Research Group study (AREDS, 2001a) and is based upon the aforementioned clinical appearances.

A. **No AMD** (AREDS category 1). No or a few small drusen (<63 microns in diameter)

B. **Early AMD** (AREDS category 2). Any or all of the following: multiple small drusen, few intermediate drusen (63 to 124 microns in diameter), or RPE abnormalities.

C. **Intermediate AMD** (AREDS category 3). Any or all of the following: extensive intermediate drusen, and at least one large druse (≥ 125 microns in diameter), or GA not involving the centre of the fovea.

D. **Advanced AMD** (AREDS category 4). GA involving the fovea and/or any of the features of neovascular AMD (AREDs, 2001a).

Dry AMD is common in the western world. Estimates for the UK population are a prevalence of late AMD of 4.8% (95% CI 3.4% to 6.6%) of those over

65 years of age and 12.2% (95% CI 8.8% to 16.3%) of those aged 80 years or more in the UK (Owen et al, 2003).

Those affected may experience no visual symptoms in the early phases of dry AMD. Difficulty in reading is a typical early symptom with people requiring more light and having a reduced tolerance. Symptoms usually progress slowly over a period of many years. Late stage symptoms are characterized by central scotomata (holes in the vision), making tasks such as reading and the recognition of facial features difficult.

The diagnosis of dry AMD is a clinical one, with typical changes being observed directly by ophthalmoscopy. It is usually a bilateral disease, although progress may be asymmetrical (AREDS, 2001a).

Although inhibitors of the pro-inflammatory complement pathway have shown promise to slow the progression of GA in early phase clinical studies (Do et al, 2014), currently there is no effective routine clinical treatment of dry AMD. Vitamin supplementation in the form of high dose combination has been shown to reduce the conversion of 'dry' to 'wet' disease however (AREDS, 2001b).

1.2.1.1.2 Neovascular Age-related Macular Degeneration

Neovascular (new blood vessels) AMD, also termed 'wet' AMD, is a less common but potentially more devastating form of the disease. The incidence has been estimated at 450 per million in the UK (Owen et al, 2003). It is typically of unilateral onset with the second eye becoming involved in approximately 25-42% after 5 years (AREDS, 2001a). Neovascular AMD (nvAMD) is of three principle types.

A. **Choroidal Neovascular Membrane.** A choroidal neovascular membrane (CNV) occurs when there is a breach of Bruch's membrane, the tissue directly beneath the RPE, and a fibrovascular network of blood

vessels arises from the choroidal circulation. A breach of Bruch's membrane is not unique to AMD. It also occurs in trauma, shortsightedness and hereditary abnormalities of its constituent collagenous tissue such as pseudoxanthoma elasticum, but dry AMD is the commonest cause. The CNV enters either the sub RPE space, the sub retinal (neurosensory) space or both. These blood vessels are abnormal; being highly fenestrated they leak serum and lipids, and are prone to haemorrhage (Kanski, 1999). Fluid can accumulate beneath the RPE, the neurosensory retina, within the retina and cause the RPE to detach from the underlying Bruch membrane (a so called 'Pigment Epithelial Detachment' or PED).

B. **Retinal Angiomatous Proliferation**. A Retinal Angiomatous Proliferation (RAP) lesion is a similar neovascular abnormality to CNV but the lesion arises from within the retina (stage 1). The lesion may extend to below the neurosensory retina (stage 2) and in the late stage of the disease (stage 3) it anastomoses with the choroidal circulation when CNV then becomes present (Yannuzzi et al, 2012).

C. **Polypoidal Choroidal Vasculopathy.** Polypoidal Choroidal Vasculopathy (PCV) is also a form of wet AMD usually occurring at a slightly earlier age and more nasal in the macula than CNV, and typically with a blood filled 'serosanguinous' PED. PCV is characterized by an arborizing network of choroidal blood vessels, with dilated vessels that are seen as 'polyps'. These do not often enter the sub retinal space and are only associated with CNV in approximately 10% of cases (Yannuzzi et al, 1997).

Wet AMD causes symptoms of acute loss of vision, often accompanied with distortion of lines that would otherwise be seen as straight, a symptom known as metamorphopsia. The natural history of wet macular degeneration is poor due to haemorrhage and fibrosis (scarring) ultimately disrupting the function of the retina. This typically leads to severe loss of central vision and blindness over a period of many months (Rosenfeld et al, 2006).

Diagnosis of nvAMD relies on clinical judgment of typical features (AREDS 2001a) at ophthalmoscopy supported by investigations. Fundus Fluorescein Angiography (FFA) is required to confirm the presence of CNV, whilst Indocyanine Green Angiography (ICG) confirms PCV. Both of these techniques are dynamic examinations of the circulation, FFA of the retinal and ICG of the choroidal systems. Following injection of the fluorescein or Indocyanine Green dye into a vein in the arm, photographs are taken of the posterior aspect of the retina (the Fundus) over a 10-15 minute period. Two main forms of CNV are seen on FFA. Classic lesions, forming about 15 % of all CNV, are well defined in the early stages of the angiogram (within 20 seconds), often have a lacey pattern and demonstrate leakage in the later stage. Occult lesions are ill defined, are often described as having a 'speckled' hyperfluorescence, and leak in the later stages. Commonly a combination of the two patterns is observed and lesions may be described as 100% classic, predominantly classic (>/= 50%, Figure 1), minimally classic (<50%) or occult. A third rare description of 'late leakage of undetermined origin' has been made, whereby leakage is not seen until 2 minutes or more after the dye has been injected (Macular Photocoagulation Study, 1991).
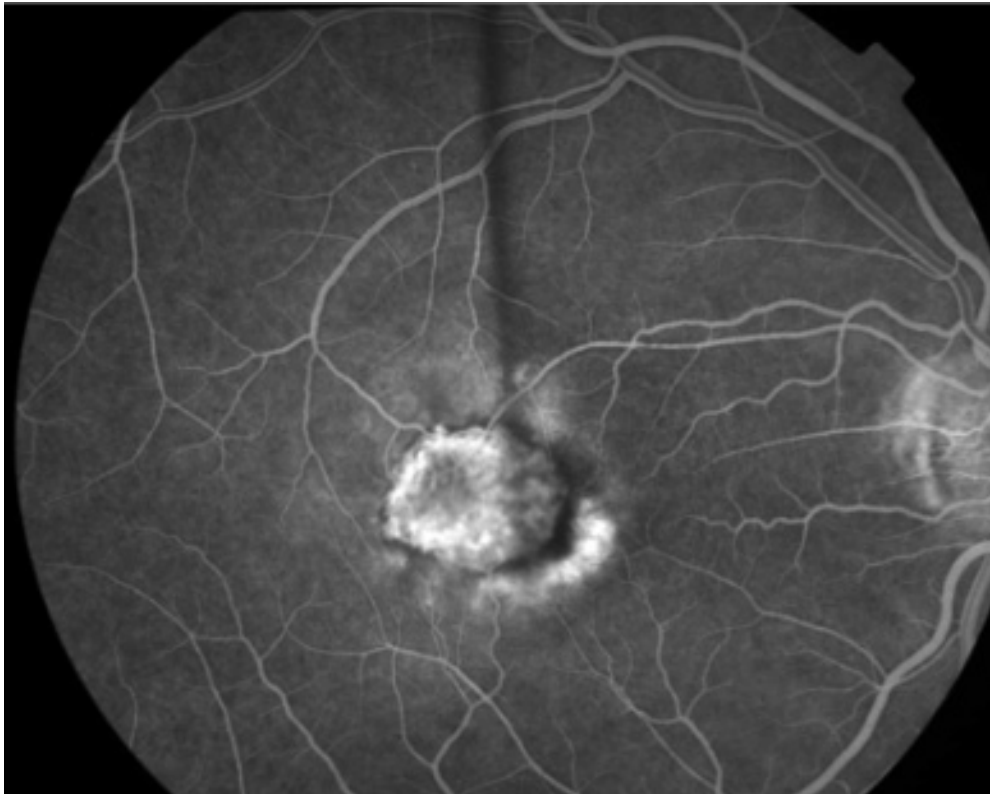
Figure 1. A Fundus Fluorescein angiogram demonstrating a predominantly classic Choroidal neovascular membrane. This particular image of the FFA is in the arterio-venous phase (the dye is in both arterioles and veins). It demonstrates a well defined hyperfluorescent area (white) with dark pigment encircling it (the classic component) with a less well defined hyperfluorescent area on the lower right border demonstrating the occult component.

Optical Coherence Tomography (OCT) is a non-invasive method of evaluating the retina used to complement the techniques of FFA and ICG. OCT is a non-dynamic examination of the central macular region demonstrating the retinal and to a lesser extent adjacent choroidal architecture. OCT is based on the principle of analysis of reflected waves of laser light to from a 2-dimensional 'A-scan'. Tissue interfaces reflect the light allowing anatomical structures to be defined. A 3-dimensional image is formed when multiple A-scans are taken to form a 'B-scan'. Using

Fourier/spectral domain technology an image comprising of a few thousand cross sections can be performed in a few seconds (Figure 2).
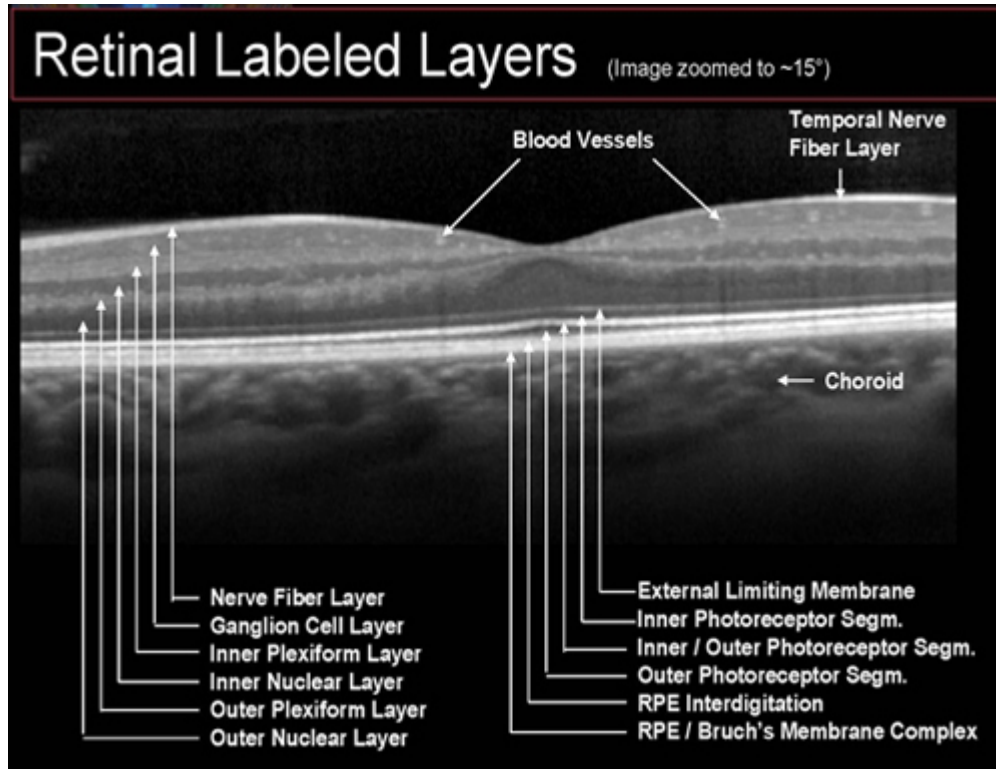
Figure 2. A spectral domain OCT image of normal macula anatomy. From http://586eyes.com/diagnostic-center/spectralis/. Accessed 23 September 2015

1.2.1.1.2.1 The Treatment of Neovascular Age-related Macular Degeneration

The treatment of nvAMD has rapidly evolved over the last 15 years with particular advancement during the last 8 years.

One option for the treatment of CNV is thermal (or 'hot') laser therapy. Applied directly to the lesion the energy cauterizes the vascular network and so prevents further neovascularisation. Significant collateral damage to

the associated retina and RPE occurs, effectively destroying the tissue and leaving a hole in the vision, a 'scotoma'. Whether this scotoma is important or not depends on its location. If the laser is applied to a CNV in an extrafoveal location (that is >200μm from the centre of the fovea) then the induced scotoma may not be symptomatic as it leaves the photoreceptor/RPE complex required for functional vision unaffected. If the laser treatment is given in a juxtafoveal location (1-200μm) there is a higher chance of symptoms. If the laser 'burn' is subfoveal there is inevitably a sudden reduction in vision at the time of laser treatment as the central cone photoreceptors are destroyed. In this situation the resulting visual loss is often worse, at least initially, than the visual loss due to the CNV itself (Macular Photocoagulation Study, 1991). Thermal laser is now only recommended for some extrafoveal lesions when other forms of therapy may not be in the best interests of the individual.

Photodynamic therapy involves targeting the CNV with a different wavelength of ('cold') laser to try and minimize collateral damage. An 'exciting' agent, verteporfin, is injected into the systemic circulation. After 83 seconds it accumulates in a higher concentration in the choroidal rather than the retinal circulation. The applied laser then induces a photochemical effect treating the CNV but minimizing damage to the surrounding retina. Although this had a statistically beneficial effect on reducing the speed of visual loss due the CNV, translated into clinical effect the results were often disappointing (TAP study, 1999).

The major breakthrough in treatment of nvAMD occurred when for the first time a treatment became available that enabled some restoration and stabilisation of vision. Vascular Endothelial Growth Factor (VEGF) is one of the principle chemical transmitters that promotes the growth and leakage of blood vessels. It is found in a particularly high concentration in association with neovascularisation. Anti-Vascular Endothelial Growth Factor (anti-VEGF) agents are commonly used to treat new blood vessel growth in cancer often being used as adjunctive chemotherapeutic agents. A trial of

intravenous injection of bevacizumab (trade name Avastin, Genentech Inc. USA) in 9 patients with nvAMD demonstrated an improvement in visual acuity, reduction in leakage of CNV on FFA imaging and thinning of the retina using OCT (Michele, Rosenfeld, Puliafito, Marcus & Venkatrman, 2005). A subsequent trial of injection of a small volume of bevacizumab into the vitreous body via the pars plana demonstrated resolution of subretinal fluid and visual improvement (Rosenfeld, Moshfeghi & Puliafito, 2005). In parallel to this 'off-label' use of bevacizumab, ranibizumab (trade name Lucentis, Genentech Inc. USA) was being developed from just the monoclonal antibody fragment of bevacizumab, as a treatment specifically licensed for nvAMD. Ranibizumab is a humanised recombinant monoclonal antibody fragment targeted against human vascular endothelial growth factor A (VEGF-A). It binds with high affinity to all the VEGF-A isoforms thereby preventing binding of VEGF-A to the receptors VEGFR-1 and VEGFR-2.

Landmark clinical trials (Brown et al, 2006; Rosenfeld et al, 2006) using fixed monthly injection of ranibizumab demonstrated a mean gain in visual acuity after 3-4 injections with relative stability thereafter. Ranibizumab (marketed by Novartis Pharmaceuticals AG in Europe) was licensed in Europe in 2006 and approved for routine use in the NHS by the National Institute for Health and Clinical Excellence in August 2008 (NICE, 2008). In the UK, due to its unlicensed nature and the NICE 2008 ruling, bevacizumab is rarely used, but in many other countries it is used more commonly than ranibizumab. It is worth noting that the same company, Genentech, manufactures both agents. The fact that Avastin, at the volume required for the eye, is significantly cheaper than ranibizumab and is not recommended for the use in the eye by Genentech has led to much controversy. Independent comparative trials for efficacy have led many commentators to describe similar efficacy with possible minor differences in systemic safety signals at a population level between the agents. The debate continues. (Martin, Maguire, Ying, Grunwald, 2011; Chakravarthy, Harding, Rogers, Downes, Lotery, Wordsworth, et al 2012).

In July 2013 the molecule aflibercept (trade name Eylea, Bayer HealthCare) was approved by NICE for use in nvAMD. Aflibercept is a fusion protein designed to bind multiple isoforms of Vascular Endothelial Growth Factor-A (VEGF-A) and Placental Growth Factor (PlGF). PlGF is another protein involved in the abnormal growth of new blood vessels. Visual acuity results have been similar to those in the clinical trials studying ranibizumab, but dosing is different. Both products are given monthly for three doses then aflibercept is given 8 weekly for the first year rather than the monthly as required schedule for ranibizumab (Schmidt-Erfurth et al, 2014).

It has become clear that anti-VEGF agents, for all their ability to restore some vision do not provide a cure for neovascular disease. By in large treatments simply suppress disease until the clinical effect is no longer apparent and treatment is re administered. Consequently the majority of those with neovascular disease need to be monitored for signs of disease activity and treated in the long term (Rofagha et al 2013; Tufail et al, 2014; Airody, Venugopal, Allgar & Gale, 2014).

The effect of introduction of anti-VEGF agents for treatment of neovascular AMD has been truly remarkable: the burden of blindness has reduced by approximately 50% over the last decade (Bloch et al, 2012).

1.2.1.2 The Pathogenesis of Age-related macular degeneration

The pathogenesis of AMD is multifactorial. It can be thought of in terms of predisposing factors, and triggers / drivers of the disease ultimately leading to chronic destructive inflammation (Table 2). A number of candidate genes have supported that notion that there is a strong genetic component predisposing to AMD. Complement factor H, being one of this first major genes to be identified with a mutation in C3, confers a 2.6 times greater risk of developing the disease (Thakkinstian, et al, 2006). The complement pathway is one of the naturally occurring pro inflammatory pathways.

Oxidative stress is the key mechanism in the development and progression of the disease through the production of free radicals and ultimately chronic inflammation of the retinal tissues. Oxidative stress occurs through disease triggers and drivers such as smoking, hyperglycemia, poor vascular disease, as well as the age-associated accumulation of lipofuscin by-products of the photoreceptor transduction cycle. As we age naturally occurring anti-oxidants in the macula, such as the carotenoid pigments lutein and zeaxanthin, are less able to protect against these harmful stresses. The end result of all of these factors is an increased production of chronic inflammation with a reduced ability to be able to keep this in check (Pujol-Lereis, SChlafer, Kuhn, Rohner, Pauly, 2016)

Pathogenesis of AMD	
Predisposition	Genetic mutations e.g. Complement factor H
Triggers/ Drivers	e.g. Smoking Hyperglycemia Lipofuscin accumulation
Retinal Destruction	Chronic Inflammation

Table 2. A summary of the pathogenesis of Age-related Macular Degeneration

1.2.1.3 Myopic Macular Degeneration

Pathological myopic degeneration is the second commonest form of macular degeneration. It is diagnosed by characteristic ocular appearances in patients with at least 6 Dioptres of Myopic correction. It affects approximately 2–4% of Caucasians and 9–21% of Asian populations (Montero & Ruiz-Moreno, 2010; Soubrane, 2008). In a similar way to AMD it can be divided into dry (atrophic) and wet (neovascular) forms.

1.2.1.3.1 Atrophic Myopic Degeneration

A characteristic feature of dry degeneration due to myopia is peripapillary (adjacent to the optic disc) atrophy of the retina and RPE. This can progress to affect the central macula and fovea causing profound visual loss.

1.2.1.3.2 Neovascular Myopic Degeneration

The central vision loss that can occur in patients with pathological myopia (PM) most commonly results from CNV. It occurs in 4–11% of affected eyes and predominantly in those younger than 50 years of age. The natural history dictates that almost 90% of eyes will develop severe visual loss (20/200 or less) after 5–10 years (Yoshida, Ohno-Matsui & Yasuzumi, 2003). The chance of the fellow eye being affected is high with around a third being affected within 8 years (Ohno-Matsui, Yoshida & Futagami, 2003).

1.2.1.3.2.1 The Treatment of Neovascular Myopic Degeneration

In a similar manner to Laser based therapies for CNV associated with AMD, thermal laser for pathological myopia is of limited value. Thermal laser to subfoveal lesions will cause severe and immediate visual loss. Whilst this does not occur with thermal treatment of juxta- and extrafoveal lesions, expansion of the laser induced chorioretinal scar into a subfoveal location characteristically seen in myopia, and a high recurrence rate, dictates that it is rarely used. (Chan et al, 2005; Secretan, Kuhn, Soubrane & Coscas, 1997).

Photodynamic therapy using verteporfin was the mainstay of treatment until injectable treatment started being used, but at best this slowed down the progression of visual loss. Data from a large multinational, randomized clinical trial demonstrated a significant benefit for PDT over sham therapy for the primary end point of what was considered to be clinically significant vision loss (72% versus 44%; $p < 0.01$), but this effect was lost after 2 years

(64% versus 49% p=0.01) (Blinder, Blumenkranz & Bressler, 2003). Small case series using the 'off-label' intravitreal anti-VEGF agents show promise in outperforming PDT (Cohen, 2009).

1.3 Measuring outcomes of treatment of neovascular macular degeneration

Having established there are now successful treatments for neovascular macular degeneration (nvMD) is it important to be able to quantify their outcome, not only from a clinical standpoint but also from a scientific point of view. Outcomes measurements can be broadly divided into the following categories:

A. Anatomical.	A measure of anatomical restoration
B. Functional.	
i) Clinician / scientist measured.	A measure of restoration of visual function
ii) Patient / participant reported.	Patients' own perception of improvement

1.3.1 Anatomical outcomes of treatment of neovascular macular degeneration

Restoration of the anatomy of the retina, RPE and choroid to its pre diseased state is a key goal of treatment of nvMD (Brown et al, 2006; Rosenfeld et al, 2006; Martin et al, 2011; Chakravarthy et al, 2012). The principle ways of measuring the anatomy have been introduced already:

1.3.1.1 Angiography

FFA and ICG are excellent tools and are still considered the gold standard in diagnosis of nvMD. Leak of the fluorescein dye out of the abnormal vascular network on FFA is a marker of neovascular disease activity. The fluorescein or indocyanine dye is administered intravenously and although some form of abnormal reaction such as nausea, vomiting or rash is relatively common (5-10%), anaphylactic reaction leading to death is very rare (1 in 250,000). Given the invasiveness of these techniques FFA and ICG are rarely routinely used to monitor disease progress at every patient visit and therefore limits its use as a high volume tool for assessment.

1.3.1.2 Optical Coherence Tomography derived outcomes

The principles of OCT have been described above. It is a rapid, non-invasive method of measuring single time point anatomy and therefore useful when high volumes of assessments are required. Common resolution of the OCT in a clinical based instrument is 6μm (Cirrus OCT, Carl Zeiss Meditec, Dublin, CA). OCT is useful if defining normal anatomy and pathological changes such as:

A. Tissue loss (for example atrophy of the inner and outer segment junction of the photoreceptors or loss of the contact cylinder of photoreceptor / RPE junction).

B. New tissue (such as sub retinal fibrosis occurring secondary to a CNV. Fibrosis, haemorrhage and CNV all have similar reflectivity so distinguishing between these is not accurate and they are often grouped together as 'Sub Retinal Highly Reflective Material' or SHRM).

C. New spaces (which may be assumed to be fluid filled, such as intra retinal fluid or subretinal fluid associated with incompetent new blood vessels, or alternatively neuronal loss).

OCT alone does not give a dynamic assessment of anatomy and so it is not possible to distinguish whether fluid seen on an image is a stagnant, inactive 'pool' or a 'leak' denoting active disease. Given the characteristics of CNV, assumptions are often made based on previous history, findings and clinical experience. OCT is also limited by its resolution. It follows that if higher resolution technology were available more detailed anatomy and pathology could be observed, which in turn may influence treatment criteria.
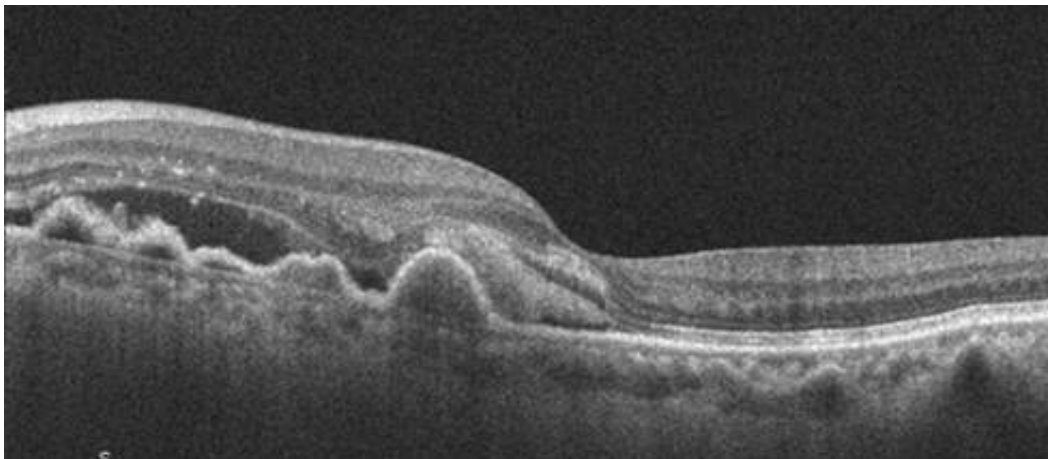
Figure 3. A Spectral domain OCT image through the central macula demonstrating neovascular age-related macular degeneration. The left side of the image demonstrates elevated areas of the RPE ('pigment epithelial detachments') and fluid (dark) beneath retina and above the RPE ('sub retinal fluid').

1.3.2 Functional outcomes of treatment of neovascular macular degeneration

Restoration of retinal anatomy does not necessarily lead to the restoration of function as 'damage' to the integrity of the tissue may occur. Anatomy may therefore not be a good surrogate marker of function. The following section introduces the techniques of visual acuity, microperimetry, functional magnetic resonance imaging and patient reported outcomes as markers of functional outcomes of treatment of nvMD.

1.3.2.1 Visual Acuity

Visual acuity is a measure of the spatial resolution of the visual processing system and is dependent of the entire visual pathway. Visual acuity is commonly measured by testing the ability to read high contrast black letters (optotypes) on an illuminated background at 6 metres, a distance that is assumed to be at infinity for optical purposes. The Snellen chart is a common example, and uses rows of letters that decrease in size down the chart. A person with normal vision would be assumed to able to see the letters on the row marked '6' at 6 metres, so called '6/6' vision. This gives an angle of resolution of 1 minute of arc. The less far down the chart is read, the higher the denominator resulting in a value that represents worse vision. The ability to read letters on a chart is influenced by a number of factors associated with the chart itself such as the contrast of the optotype compared with the background, the duration of presentation of the letters, the type of optotype and interaction effects from adjacent letters (the crowding effect of adjacent letters) (Kaiser, 2009).

The Bailey-Lovie chart uses a constant number of letters per line with a constant amount of spacing and so overcomes the issue of spacing. It uses optotype that decreases in size in a logarithmic manner and so is often termed called a LogMAR chart. In 1982 the chart was adapted using the 'Sloan' selection of letters from the alphabet, these letters all being equally recognisable, for the Early Treatment of Diabetic Retinopathy Study. The "ETDRS" chart, having 20 rows of 5 letters on each row has since been the standard for visual acuity measurement in clinical trials and gives a letter score of 0-100. (Ferris, Kassoff, Bresnick & Bailey, 1982; Ferris and Bailey, 1996).

Although visual acuity remains the gold standard assessment tool for measuring visual function, it is widely recognized that these high contrast tests of vision underestimate the level of visual impairment, particularly in older patients (Scott, Schein & West, 1994; West, Munoz & Rubin, 1997;

Mangione, Gutierrez, Lowe, Orav & Seddon, 1999; Maclure, Hart and Jackson 2000: Hazel, Petre,, Armstrong, Benson & Frost, 2000).

1.3.2.2 Retinal sensitivity as performed by Microperimetry

Perimetry is a psychophysical method of assessing retinal sensitivity. A visual stimulus is presented to a subject in a part of their field of vision corresponding to the area of the retina under examination. If they perceive the stimulus a positive response is made. The size, intensity, type (for example a light, hand movements or coloured object) and location of the stimulus can be selected depending upon the question being asked of the subject. Two broad techniques are used the map the field of vision: movement of a constant stimulus from the least sensitive peripheral field towards the centre until it is seen ('kinetic perimetry'), or the use of stimuli which are presented in a constant location but increasing in intensity until the threshold for identification is reached ('static perimetry'). An example of kinetic perimetry is the Goldmann technique. The particular importance of this technique is that the size and intensity of the stimulus it uses have set standards used with other technologies. The stimulus size varies between 0 and V (roman numerals) and intensity between 1 and 4 (for each 5dB increase), further refined by a to e for 1dB increments. (Cohen & Kawasaki, 1999). Typically I4e is used for a peripheral assessment and I2e for a central assessment.

An automated process of perimetry enables the determination of retinal sensitivity in a numerous fine locations in a reliable and reproducible manner if so required. A key aspect of perimetry is ensuring the subject maintains fixation of vision in a constant place throughout the examination and hence eye movements are kept to a minimum. Automated perimetry is most commonly used to diagnose and monitor the effect of treatment of visual field defects in glaucoma, a condition affecting the optic nerve that characteristically produces peripheral visual defects.

Microperimetry enables the determination of the map of retinal sensitivity in a much smaller field than conventional perimetry. This technique was first developed using a modified colour camera and subsequently using a scanning laser ophthalmoscope, a method using laser technology to view the retina in real time and therefore monitor the precise retinal localization of the stimulus and the ability of the patient to maintain fixation (Van de Velde, Timberlake, Jalkh & Schepens, 1990). The Nidek MP-1 Microperimeter (Nidek Technologies, Padova, Italy) uses an infrared camera in conjunction with an automated eye-tracking system that shifts the position of what should be constant stimulus locations to compensate for small eye movements. This allows for precise microperimetric assessment of central field sensitivities (Squirrel & Elrich, 2012).

Squirrel et al studied a small number of patients before and after commencing anti-VEGF treatment for nvAMD. They used the MP-1 microperimeter with 45 Goldman III (medium) sized stimuli over a 12-degree macular area. After the first 3 doses of treatment there was a mean increase of 6 (-15 to +12) ETDRS letters and 2.85 (SD 1.55) dB retinal sensitivity. Based on only 1 of 10 patients having an improvement in visual acuity but 8 of 10 having an improvement in retinal sensitivity, they concluded that visual acuity appeared to underestimate the functional improvement seen with retinal sensitivity (Squirrel, Mawer, Mody & Brand, 2010).

1.3.2.3   The BOLD response as measured by Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) is a neuroimaging technique that uses Magnetic Resonance Imaging (MRI) to measure functional changes in the brain based upon blood oxygenation (and probably volume). As neuronal activity increases there is an increase in the amount of oxygenated blood compared with deoxygenated blood. The additional oxygenated blood also enables the delivery of glucose to neurons so they can be repolarized,

i.e. returned to their ready state, following previous activity. Neuronal activity causes a release of glutamate, which ultimately leads to release of nitric oxide, a powerful vasodilator, and so increases blood flow.

Deoxygenated hemoglobin is more magnetic than oxygenated hemoglobin leading to an increase in the Magnetic Resonance (MR) signal detected. This improvement can be mapped to show which neurons (or in fact many thousands of neurons in a small volume called a voxel) are active at a particular time. This so called Blood Oxygenation Level Dependent (BOLD) contrast was taken advantage of by Ogawa in 1990 who pioneered, initially in rats, the now commonly used research technique of fMRI (Ogawa, Lee, Kay & Tank, 1990). The BOLD contrast can be quantified and the three dimensional area that is studied divided into component parts or voxels to show detailed functional change in specific anatomical locations: Functional Magnetic Resonance Imaging is able to localise BOLD to within 2-3 mm of the neuronal activity.

The increase in oxygenation typically lags 1-2 seconds behind neuronal activity and peaks at 5 seconds. Once the stimulus for brain activity is removed the level falls, overshoots below its original value slightly before returning to normal.

Functional MRI is affected by unwanted signal, termed noise, from the scanner and random brain activity, which can be as big as the signal itself. To minimise this, fMRI studies repeat a stimulus presentation multiple times and a mean response is taken.

Give its high retinal sensitivity the macula has a relatively large representation in the visual cortex compared with the peripheral retina. It is represented at the posterior pole of the cerebral cortex within the calcarine sulcus. This Region Of Interest (ROI) can be pre-defined on an anatomical MRI scan for study and signal change within this area can be studied when the macula is stimulated with light. The amplitude and coherence of change

of the fMRI activity, synchronized with the stimulus can be calculated (Baseler, 2011a).

Baseler et al studied a single case and reported improvement in fMRI signal in a patient treated with ranibizumab for neovascular Age-related Macular Degeneration. Functional MRI was suggested as a sensitive and objective measurement of visual function as it does not rely on the patient to maintain good visual fixation that can interfere with techniques such as VA and microperimetry. Furthermore, the cortex appeared to remain responsive when vision was restored (Baseler, 2011a).

1.3.2.4 Patient reported outcomes

Patients can report their own perception of response to treatment. A number of different Patient Reported Outcomes (PRO) specific to eye disease or treatment exist enabling a structured response that individuals perceive to be recorded.

The National Eye Institute Visual Function Questionnaire (NEI VFQ) is an example of a commonly used Patient Reported Outcome Measure (PROM) (Mangione, Berry & Spritzer, 1998). It was initially developed as a 51-point scale that sampled different aspects of visual function with the help of patients. (Mangione et al, 1998). It was further refined to a 25 point scale, which correlates well with the 51-pont scale and still being representative of visual function (Mangione et al, 2001). The NEI VFQ has been used to provide insight into the quality of life change following anti-VEGF treatment for nvAMD (Rakic et al, 2013).

The 12-item well-being questionnaire (W-BQ12), (Riazi, Bradley, Barendse, & Ishii, 2006) was developed from the longer 22-item version and comprises 3 areas of assessment: Energy, positive well-being and negative well-being (Bradley & Lewis, 1990; Bradley 1994). Each of these 3 areas has a score of 12 points, making a total of 36 possible and a higher score

indicates a better well-being. Although the PROM was developed for use in patients with diabetes, it has been shown to be a useful and reliable tool in patients with macular disease (Mitchell & Bradley, 2001).

The treatment satisfaction questionnaire MacTSQ (Mitchell, Brose & Bradley, 2007) was designed to assess treatment satisfaction in two areas: impact of treatment, and provision of information and convenience. Each of these scales can score a maximum of 36 points, making a potential maximum score of 72 with a higher score indicating better satisfaction. The MacTSQ was used in the IVAN trial, the results of which are awaited (Chakravarthy et al, 2013).

1.3 Measuring the adverse effects of treating nvAMD

All treatments have side effects and anti VEGF therapy of nvAMD is no exception. Side effects can be broadly considered as effects of the pharmacological agent and adverse effects of the procedure.

1.4.1 Adverse effects of the pharmacological agents

There has been much debate about the side effects of suppressing VEGF, particularly about potential systemic complications (Chakravarthy et al 2012, Martin et al, 2011). The SAILOR clinical trial studying the safety of ranibizumab therapy in nvAMD, warned of an increase in cardiovascular and cerebrovascular endpoints such as myocardial Infarction, arrhythmia and stroke (Boyer, Heier, Brown, Francom & Ianchulev, 2008). Since then most studies have not convincingly proven any excess cardiovascular events but debate remains as to the possibility of slight excess of haemorrhagic stroke (Bressler et al, 2012).

1.4.2 Adverse effects of the intravitreal injection procedure

The intravitreal injection procedure can inadvertently cause retinal tears, vitreous haemorrhage or lenticular trauma. These are uncommon events occurring at a frequency of less than 1 in a 1000. Introduction of infection into the vitreous cavity, an ideal culture medium for bacteria and fungi can lead to the complication of endophthalmitis. Endophthalmitis is a severe inflammation of the ocular structures that occurs at a rate of approximately 1 in 3000 injections often having a devastating outcome on final visual acuity (Boyer et al, 2009; Hasler et al, 2014).

Typically a volume of 0.05mls of an anti-VEGF agent is administered. As the ocular structures are relatively rigid this leads to an intraocular pressure rise (Kim et al, 2008). Intraocular pressure elevation is the main risk factor glaucoma, a condition characterized by damage to the optic nerve, with loss of retinal nerve fibres and consequently peripheral vision. The role of treatment to protect glaucoma patients from short-term pressure spikes at the time of injection has not been well studied.

1.5 The Aim of this Thesis

Being able to adequately quantify the outcome of neovascular AMD treatment gives perspective not only on appropriate intervention for individual patients, but also the understanding of the science behind the disease, its therapeutics and design of future clinical trials.

Commonly used methods of measuring VA using high contrast charts often underestimate visual function (Scott et al, 1994; West et al, 1997; Mangione et al, 1999; Maclure, Hart and Jackson 2000: Hazel, Petre, Armstrong, Benson & Frost, 2000). The Study by Squirrel et al concluded that VA might underestimate the functional improvement in vision of nvAMD patients treated with ranibizumab (Squirrel et al, 2010). It would be logical to assume that there is a strong correlation between the restoration of normal

anatomy and the restoration of visual function; however this is not consistent finding (Munk et al, 2013). Baseler et al suggested that fMRI might provide a functional measurement of visual function avoiding some of the problems with VA and microperimetry (Baseler, 2011).

Concluding from this introductory chapter, there is enough discussion to warrant further study of the common methods, and development of new techniques, which assess the outcomes of treatment of neovascular macular degeneration.

This thesis aims to assess the common forms of outcome measures of treatment of macular degeneration such as visual acuity assessment and OCT imaging. It also aims to explore and develop the use the functional measures of patient reported outcomes, microperimetry and functional MRI. In line with this theme not just the positive aspects of therapy will be studied, but also a negative aspect in the form of the prevention of short-term pressure spikes following intravitreal injection. These outcome measures will be evaluated in the context of a series of observational and interventional studies assessing new and existing treatments and techniques. Conclusions about these techniques and treatments, their advantage and pitfalls as well as their appropriateness for scientific and clinical use will be evaluated.

Chapter 2. Visual Acuity as an Outcome measure of anti-VEGF treatment of Neovascular Age-related Macular Degeneration

2.1 Introduction

Following on from introducing some of the common methods of assessing outcomes of anti-Vascular Endothelial Growth Factor (VEGF) treatment of Neovascular Macular Degeneration (nvMD) in chapter 1, this chapter will study the value of using visual acuity (VA) as an assessment tool. A cohort of patients being treated with ranibizumab for neovascular Age-related Macular Degeneration (nvAMD) was studied. The cohort initially had a longer than recommended follow up interval, but as a part of redesign of clinical services this was shortened to the required interval. Visual acuity was used to assess the impact of this change.

2.2 Background

In the developed world nvAMD is the commonest cause of severe visual loss in the retired population and accounts for more than half of all cases of those registered sight and severe sight impairment in the United Kingdom (Rostron & McKibbin, 2012). The incidence of nvAMD rises with age being 0.2% at age 55-64 years and 13% over the age of 85 years and equates to an estimated 26,000 new cases in the United Kingdom per year (Bunce, Xing & Wormold, 2010; Owen, Jarrar, Wormald, Cook & Fletcher, 2012). So with the knowledge that anti-VEGF treatments by and large temporarily suppress the disease rather than providing a cure (Rofagha et al 2013; Tufail et al, 2014; Airody et al, 2014) and that we have an aging population, the prevalence of treated nvAMD continues to rise. This has led to a substantial increase in capacity demand and many nvAMD treatment clinics are failing to meet this demand (Amoaku, Blakeney, Freeman, Gale, & Johnston, 2012).

Early treatment of nvAMD with anti vascular endothelial growth factor agents in order to prevent the natural history of rapid and progressive

visual loss (Bressler, 2001) is standard care. This is evidenced by the landmark studies ANCHOR (Anti-VEGF antibody for treatment of predominantly classic choroidal neovascularisation in AMD) and MARINA (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD) (Brown et al, 2006; Rosenfeld et al, 2006). Typically there is an initial gain in mean VA of 7 to 9 ETDRS letters with subsequent stabilisation. Studies have shown that equally important to early diagnosis is regular and long-term follow up, enabling timely re-treatment as required. These phase III clinical trials have demonstrated that regular treatment by either injection on a 'fixed dosing' regimen or an 'as required' (*pro re nata* or 'prn') regimen, with ranibizumab on a monthly basis leads to long term visual stability (Boyer et al, 2009; Brown, 2006 et al; Martin, Maguire, Ying, Grunwald, 2011; Martin et al 2012; Lalwani, Rosenfeld, Fung, Dubovy, Michels, 2009; Rosenfeld et al, 2006). However, fixed dosing at a quarterly interval following three initial monthly ranibizumab injections leads to a gradual decline of the initial gain in visual acuity (Regillo et al, 2008). Furthermore, the monthly regime studies also demonstrated less frequent severe visual loss (defined as greater than or equal to 15 ETDRS letter loss) and more significant visual gainers (defined as greater than or equal to 15 ETDRS letter gain) than either no treatment or treatment with photodynamic treatment (Brown et al, 2006; Rosenfeld et al, 2006). Mean visual acuity change and percentage of 15 ETDRS letter gainers or losers are two common parameters measure when evaluating treatments of vision threatening disease (Bressler, 2001; Brown, 2006; Rosenfeld, 2006).

With the knowledge that loss of vision is the consequence of not seeing patients in a timely manner and that long-term therapy is required, many nvAMD treatment centres have needed to reconfigure services to be able to cope with continually rising demands. There are well-documented solutions of how this may be achieved including 'off site' closer to home models. (Amoaku et al, 2012). In order to reduce follow up intervals in one nvAMD

clinic (York Teaching Hospital, United Kingdom), a community eye clinic was established (The Eye Site Clinic, Bridlington, UK).

2.3 Aims

The aim of this study was to evaluate the impact of reducing follow up intervals in a cohort of individuals with nvAMD being treated with ranibizumab, and evaluate the use of visual acuity as an outcome measure.

2.4 Study Design

The National Institute for Health and Clinical Excellence (NICE) Technology Appraisal (TA) 155 for nvAMD recommends that patients are treated with 3 monthly initiation doses of ranibizumab, 0.5 mg, as part of the standard induction phase (NICE, 2008). Patients should then be reviewed monthly and retreated if there are signs of disease in an 'as required' phase (*pro re nata* or *prn*). All patients at the 'base' hospital (York Teaching Hospital, UK) were routinely managed in this way with signs of disease activity being similar to that used in Martin et al's Comparative Anti-VEGF Treatment Trial (CATT): reduced VA explained by nvAMD, presence of intra or sub retinal fluid on Ocular Coherence Tomography (OCT) or leakage on FFA (Martin et al, 2011). Due to capacity limitations assessment intervals beyond the 3 initiation doses were often more than recommended.

To help address the shortfall in capacity and enable care to be given closer to home 154 patients with 189 treated eyes were identified and had their care transferred from the base hospital (BH, York Teaching Hospital, United Kingdom) to the community eye clinic (CEC, The Eye Site Clinic, Bridlington, UK). The new facility enabled a strict 28-day follow up interval to be offered to all patients.

To be eligible for the study patients had to be at least 50 years of age and have and a fluorescein angiogram confirmed diagnosis of nvAMD. In addition to this, the patients must have been in the 'stability phase' of their treatment defined as one month after having completed their 3 initiation treatments with ranibizumab. These three treatments must have been given at the BH each with an interval of between 28 and 35 days, this range enabling some routine flexibility in appointments. At the time of the commencement of the study the CEC had been operating for approximately 6 months enabling 6 visits at this location to be studied. To equate this study period and so to be included in the study, patients must have attended at least 12 consecutive visits in their stability phase comprising of 6 visits at the BH followed by 6 visits at the CEC. It was these visits that formed the study period.

Some treatment centres perform the assessment at one visit and if treatment is required bring patients back for treatment on a different day. This so called 'two-stop' method enables better capacity planning for the host organization but is less convenient for patients and causes further delay in the treatment pathway. Both the BH and the CEC used a 'one stop' model enabling assessment and re-treatment to be performed at the same visit.

To be consistent across sites, both locations used routine clinical VA measurement with the same type of standard ETDRS vision chart (Precision vision; Ferris & Bailey, 1996). Vision was measured in the affected eye with the patient's current spectacle correction to give Best Corrected Visual Acuity (BCVA). Consistent with routine clinical practice if the spectacle correction was considered out of date then pinhole visual acuity was used. Vision was measured at 4 metres in an illuminated room. If the patient was able to read 4 or more of the five letters on the first line they were asked to continue reading down the chart until they could read a fewer than three letters on a single line. At this point the total number of letters read correctly on the chart was recorded. Given that the measured distance was

at 4 metres then an additional 30 letters was added to the score to give a total ETDRS letter count. If the patient was unable to read 4 letters correctly on the first line they were brought to 1 metre and were asked to continue reading down the chart until they could read fewer than 3 letters on a single line. No additional 30 letters were added in this circumstance. This total ETDRS score is routinely used as a clinical measurement and was the value used for the study. Although not using a refracted score, this technique is similar to that used for many clinical trials such as the REPAIR study (Tufail et al, 2013b).

An important point to discuss is that although an ETDRS letter score is not technically a measure of logarithmic acuity (it would need a multiplying by a quotient of 0.02 to give an equivalent), its use is commonplace and standard practice for clinical trial use since it 1996 (Ferris and Bailey, 1996)

To ensure consistency between the BH and CEC, the same model of OCT, the key piece of technology helping to determine disease activity and therefore governing retreatment decisions, was used at both sites (Cirrus HD OCT 4000, Carl Zeiss Meditec).

In addition to ETDRS visual acuity, the number of injections and the time interval between visits (in days) were collected. A paired t-test was used to compare the mean BCVA, total number of injections and mean follow-up time between sites. The BCVA at each visit within each centre was compared with a repeated measures ANOVA. The proportion of patients who gained and lost 15 ETDRS letters (a figure considered to be 'significant' (MPS, 1991) between sites was compared with a chi-square test. The data analysis was undertaken on SPSS (v18, Chicago: SPSS inc.). A p-value of <0.05 was considered to indicate statistical significance.

2.5 Results

After reviewing the records of the 154 patients 72 eyes of 62 patients met the criteria to be included in this study. There were 45 female eyes and 27 males eyes with mean age of 82.0 years with a range of 60 to 96 years.

The mean follow up time between each visit in the period of study was 56.8 days (range 21 to 288 days) in the base hospital and 31.8 days (21 to 139) days in the community eye clinic. The mean difference in the follow up intervals between the base hospital and the community eye clinic was 25.0 days (SD: 14.95) and was significant (t 71=14.08, p<0.0001).

Taking a mean of all the 6 visits in the base hospital the BCVA was 54.49 ETDRS letters (SD=14.02) and 55.69 ETDRS (SD=15.49) letters in the community clinic. This mean gain of 1.19 ETDRS letters (SD=5.57) after being moved from the BH the CEC was not significant (p=0.073). In the base hospital there was a trend of the BCVA to reduce however this did not reach significance over the 6 visits (-1.13 letters; repeated measure test: p=0.871), however in the CEC there was a significant increase in BCVA over the 6 visits (+4.61 letters; p<0.001). This change is illustrated in Figure 4.
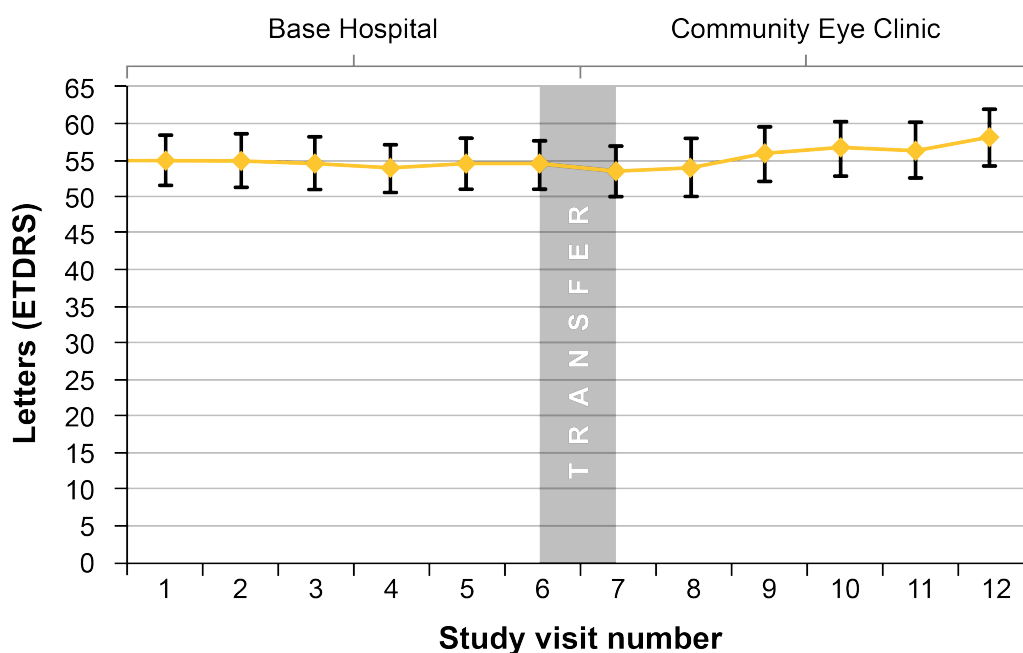
Figure 4. Mean visual acuity with 95% confidence intervals at the 6 visits in the base hospital and the 6 visits in the community eye clinic. The shaded area (TRANSFER) represents the time between the last visit at the base hospital and the first visit at the community eye clinic.

The amount of significant visual gainers (15 ETDRS letter or more) was statistically greater in the CEC compared with the BH, but the smaller number of patients experiencing severe visual loss (15 ETDRS letters or more) did not reach significance. In the BH only 1 eye out of the 72 eyes (1.3%) had a gain of 15 ETDRS letters during the first six visits compared with 9 eyes (12.5%) in the community eye clinic (p=<0.001). Seven eyes (9.7%) lost 15 ETDRS letters in the base hospital compared to 3 eyes (4.1%) in the community eye clinic (p=0.170).

The mean number of ranibizumab injections in the BH was 3.69 and 3.39 in the CEC. This was not significantly different (p=0.7). The mean percentage of patients requiring an injection at the BH was 62% and at the CEC 56% (Figure 5). The study period was not the same duration in the BH and the CEC and so adjusting for this, the predicated mean number of injections over an equivalent 6-months period assuming the same injection rate and follow up intervals, was 2.37 at the BH and 3.9 at the CEC.
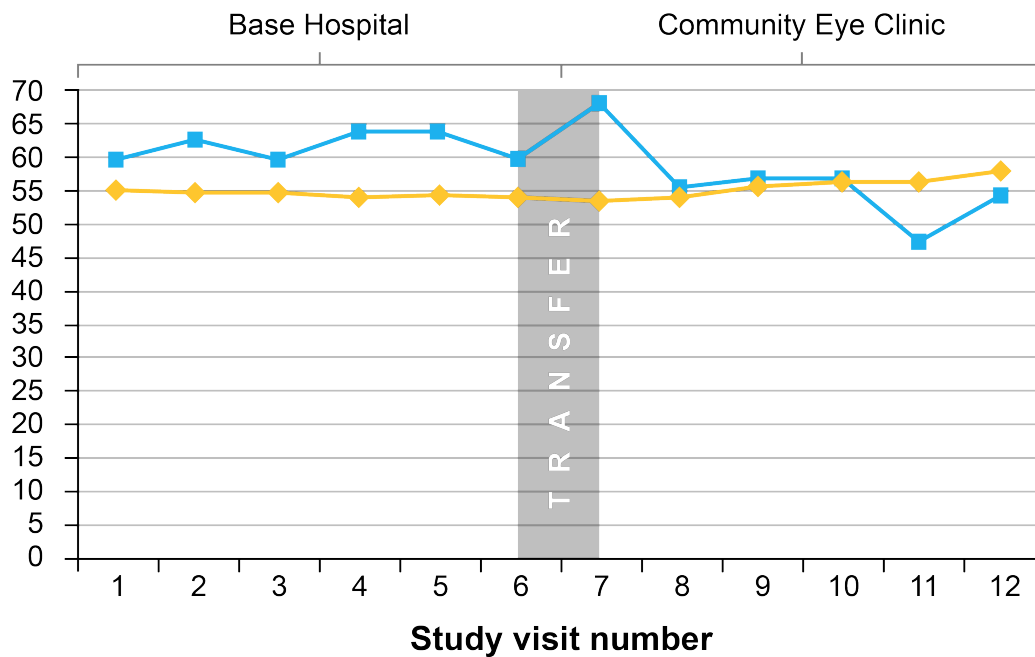
Figure 5. The percentage of patients receiving a ranibizumab injection for active nvAMD as a proportion of total assessments per month. For comparison the mean ETDRS visual acuity is plotted in the same axis. The shaded area (TRANSFER) represents the time between the last visit at the base hospital and the first visit at the community eye clinic.

2.6 Discussion

2.6.1 The implications of the Visual Acuity results

The mean change in VA in anti-VEGF treatment-naïve patients demonstrates a very characteristic response curve (Brown et al, 2006; Rosenfeld et al 2006). It can be considered to have an 'initiation phase' whereby there is a rapid gain in VA in response to the first 3 treatments, followed by a 'stability phase' during which the VA remains stable or at least there is not such a dramatic change with monthly or *prn* dosing.

The ANCHOR and MARINA studies provide good examples of this stability phase, in which there was a only a slight change of +1.3 letters between month 3 and 12 when fixed monthly dosing was used (Brown et al, 2006;

Rosenfeld et al, 2006). In the large randomized study of the use ranibizumab or bevacizumab, The Comparison of AMD Treatment Trial (CATT), a 2.4 letter gain in the fixed arm and 1.2 letter gain in the *prn* arm was demonstrated in the equivalent stability phase (Brown et al, 2011).

It is important to distinguish between the initiation and stability phases because the VA is influenced by different factors in these different phases. During the initiation phase the gain in VA is considered to be primarily a response to treatment. It is however, influenced by both patient and treatment factors. Patient factors include CNV lesion type, (as defined by angiography and demonstrates a greater increase in VA with classic lesions rather than occult ones) (Brown et al 2006, Rosenfeld et al, 2006), and delay in presentation (which may lead to more irreversible structural changes such as fibrosis) (Kelly & Barua 2011). In fact delay is considered so important on the final outcome the Royal College of Ophthalmologists have developed guidelines recommending that time from presentation to treatment should be no longer than 2 weeks (RCOphth, 2007). Ironically treatment of CNV lesion very early in their evolution may lead to a ceiling effect of VA gain: a patient with near normal starting VA will not have the same potential to gain as many letters as a patient with significantly reduced VA (Ross et al 2014). Treatment factors include the type and frequency of anti-VEGF used (slight numerical difference between ranibizumab and bevacizumab seen in the CATT trial, and between ranibizumab and aflibercept in the VIEW study) (Martin et al, 2011; Schmidt-Erfurth et al, 2014).

The VA change within the stability phase can be considered as a marker of disease control. Like the initiation phase outcomes, it is also influenced by patient factors such as lesion type (Brown et al, 2006; Rosenfeld et al, 2006) and smoking, (Klien, Knudtson, Cruickshanks. & Klein 2008). Treatment factors are also particularly important, such as the regularity of follow up and how much tolerance of signs of activity of the nvAMD is allowed. The

monitoring of VA in the stability phase can therefore reflect the quality of a wet macular degeneration service. The results of this study support this statement: there was an improvement in 4.61 letters over a 6 month period when the mean time interval between assessments was reduced from approximately 8 weeks to 4 weeks and so demonstrated an improvement in the quality of the service. The number of individuals with severe loss of vision can also be a marker of the quality of a service as discussed by Kelly in his review of safety incidents for vascular endothelial growth factor inhibitors, in which he reports 43% of incidents, mainly of severe visual loss, were due to delay in treatment (Kelly & Barua, 2012). Our study demonstrated fewer individuals losing 15 letters of more (7 compared with 3), although this did not reach statistical significance. 'Significant' gain in VA also reflects the quality of a service; in our study a gain of 15 ETDRS letters in the 2 periods of study was more in the CEC than the BH (9 versus 1).

The results of this study reflect conclusions that can be drawn from other studies that have discussed the importance of the follow–up or retreatment interval. Dagostar reported in a retrospective interventional cases series of 131 patients, that eyes receiving injections less than 2 months apart gained a mean of 2.3 lines of vision at month 6 compared with those receiving injections less frequently only gaining 0.46 lines. Similarly 3.1% of the frequent group experienced severe visual loss compared with 15.9% of the less frequent cohort (Dagostar, Ventura, Chung, Sharma & Kaiser, 2009). The retrospective nature of this study, like ours, is open to bias but adds to the argument that fewer treatments result in worse VA. The prospective randomized controlled design of the PIER study, showing declining VA in the stability phase with a mean loss of 0.2 letters from baseline at 12 months, when quarterly injections of ranibizumab following 3 initiation treatments are given adds further weight to this argument (Regillo et al, 2008).

The results of our study revealed a surprisingly large range of follow up intervals in both groups. The reasons for this included patients' wishes, failure to attend and administration errors.

The percentage of patients requiring an injection per visit was reduced when the follow up interval was reduced to every 4 weeks. The predicted total number of injections over an equivalent 6-months period however, was higher at 3.90 in the CEC compared with 2.37 at the BH. This finding that a higher injection rate leads to a higher VA is again in keeping with Dagostar's finding. (Dagostar et al, 2009).

Although this study is open to bias due to different members of the clinical staff performing assessments this bias is limited due to each member of staff having undertaken training by the same team and working cross-site. To draw a more definitive conclusion on the benefit of following individuals at either 4 or 8 weekly intervals a prospective trial randomising to these groups after 3 initiation doses would be required. The cost involved in such a study and the realisation that the results would be non 'real world' data would have to be taken into consideration if such a study were to go ahead.

2.6.2 The use of visual acuity as a measurement tool

Visual acuity is a measure of the spatial resolution of the visual processing system and is dependent of the entire visual pathway. The commonest way of measuring visual acuity in the clinical setting is with an optotype chart, usually of the Snellen type. The Early Treatment Diabetic Retinopathy Study (ETDRS) chart is now the recommended standard for measuring visual acuity in the clinical trial setting (Ferris, Kassoff, Bresnick & Bailey, 1982; Ferris & Bailey, 1996). Most clinical studies now use this modified logarithmic chart, which has Sloan letters equally spaced across the chart and 5 letters per line (Bressler et al, 2001; Brown et al, 2006; Rosenfeld et al, 2006; Martin et al 2011, Martin et al 2012).

The use of visual acuity as a measure of vision has many advantages and disadvantages. The principle advantages of the ETDRS chart are that it is a relatively quick and easy way of estimating visual performance, typically

taking 2-3 minutes in a literate individual and being performed by a member of staff that requires relatively little training. In addition a letter score gives a numerical value making descriptive and inferential statistics possible. It is possible therefore to perform power calculations to set endpoints and to perform statistical evaluations of interventions in clinical trials.

Care should be given to measure Best Correct Visual Acuity (BCVA); the best possible measured vision with the use of spectacle correction if necessary. Most high quality clinical trials that use VA as an endpoint mandate refraction (that is the measurement for glasses) at each point of VA assessment (Brown et al, 2006; Rosenfeld et al 2006; Martin et al, 2011). Refraction is not routinely performed at each visit in clinical practice though, as it takes approximately 20 minutes of an optometrist's or ophthalmologist's time. It follows on that 'real world' studies do not use refracted VAs at each visit. In real world studies if the subject has recently been refracted then this prescription should be worn. In the unusual event when the subject has forgotten their glasses the use of pinhole vision can be tried. Looking through pinholes corrects some refractive error but can make measured vision worse in people with macular disorders so may be no better than unaided vision (Walker, Hall and Hurst, 1990).

Patients with vision on the borderline of being able to use the ETDRS chart at 4 metres, that is whether they can see 4 letters on the first line and or cannot and so have to be moved to 1 metre, often demonstrate widely fluctuating measured VA on successive visits when there is no symptomatic or anatomical evidence for this to be so. Experience from this study, and clinical practice, shows that when the 1 metre chart is used measured vision is often substantially worse and can therefore appear to 'fall of a cliff'. This phenomenon requires further investigation, but may be explained by the additional effort, either on the part of the subject or the person assessing the VA, that is required to read more letters on the 1 metre chart to achieve a lower VA score than would be required for a higher score on the 4m chart.

This 'cliff' effect could artificially magnify the gain in visual acuity arising from therapeutic agents and may have led to an overestimate of the proportion of patients gaining 15 ETDRS letters. As was discussed in the original description of the ETDRS chart by Ferris in 1982, the chart could have been used at 2 metres, allowing for a range of acuities more appropriate to our cohort to be captured without having change the chart distance (Ferris et al, 1982).

The testing conditions should remain constant across multiple visits: the exact testing distance from patient to chart, the luminance of the chart (to ensure that contrast is consistent) and ambient lighting (which affects pupil size and therefore the degree of aberrations).

Even with a very consistent testing environment there have been well-documented variations in measured visual acuity. Patel et al reported on 90 fellow (non-treated) eyes in patients undergoing treatment for CNV with bevacizumab. He stratified the extent of fellow AMD into early, intermediate and late categories; these categories were discussed in chapter 1. It was demonstrated, by using coefficient of repeatability (CR, a measure of test-retest repeatability defined by Bland and Altman as the mean +/-1.96 standard deviations), that a 12-letter difference was noticed over the study period of 4 measurements in 12 weeks. (Bland & Altmann, 1986) This was refined to 10 letters after excluding patients who were tested at different distances. Worse visual acuity demonstrated a worse coefficient. The coefficient was similar when measured at an interval of a week compared with the 4 weeks. (Patel, Chen, Rubin & Tufail, 2008).

	All visits (n)	Baseline and week one only (n)
Early AMD	9 (29)	9(29)
Intermediate AMD	10 (36)	8(36)
Late AMD	17 (25)	15(25)
Total	12 (90)	11(90)

Table 3. The mean coefficient of repeatability by diagnosis, from Patel et al 2008.

Recently Aslam et al at has suggested that the repeatability may even be less than this, finding a coefficient of 14.9. The test- retest period was 4 weeks on this occasion and used similar methodology to Patel of low background luminance, 'precision vision' charts with illumination tubes that had been 'burnt in' for 96 hours and measured refracted VA (Aslam et al, 2014; Patel et al 2008). This discussion about the lack of repeatability of VA measurement is not a new one; an analysis of the Macular Photocoagulation study found a standard deviation of 4.7 letters between baseline and week one in a cohort of 60 nvAMD patients (Blackhurst & Maguire, 1989).

Our study did not use clinical trial standards but 'real world' measurements. Optometrists did not perform visual acuity testing and refracted vision was not obtained, although there were as many constants as possible applied such as testing distance and background illumination; inevitably measured VA will not be as accurate as clinical trial standards. A higher degree of variability or reduced repeatability would therefore be anticipated. The coefficient of repeatability has yet to be studied in the 'real world' situation.

The reasons for the high variability between visits can be many fold: unexpected differences in the testing environment such as the vision alley and its background illumination, the subjects general health, how much the observer encourages the subject, and the visual 'cliff effect' for example. Furthermore following damage to the fovea the development of new, or

multiple or variable areas of preferred fixation (preferred retinal loci or PRL, which is different to the area of highest retinal sensitivity) could help to explain this variation (Shima, Markowitz & Reyes, 2010). The concept of developing of new PRL's in a short period of time has been disputed following a short-term study observing for the area of fixation before and after 3 treatment with ranibizumab in previously treatment–naïve patients (Gonzalez, Tarita-Nistor, Mandelcorn, Mandelcorn & Steinbach, 2011).

The documented coefficient of repeatability makes a single point-to-point visual acuity change in an individual difficult to interpret as a measure of an outcome of treatment. Therefore VA should not be used as an absolute measure in this circumstance. When cohorts are studied and mean VAs are calculated these fluctuations are decreased, and so it is appropriate to use VA as an outcome measure. The study of the cohort of patients in this chapter has demonstrated this. Furthermore, the use of mean change in visual acuity and the change in visual acuity in the stability phase is particularly useful. Mean VA is one of the commonest endpoints for clinical trials in this field. Some examples are the mean VA gain at 12 months in the 'as required' treatment arms of the CATT, IVAN and PRONTO clinical trials being reported as 6.8, 7.2, and 9.8 letters respectively (Martin et al, 2012; Chakravarthy et al, 2012; Fung et al, 2012). Similarly, mean VA is used as an outcome measure in 'real world' studies: Querques reports +9.0 at 24 months, Michalova +5.5 at 12 months, Rostos +4.6 at 13.6 months, Rothenbuhler +7.3 at 24 months, Ross +2.4 at 24 months, and Kang +7.9 at 12 months. (Querques, et al 2010; Michalova et al 2009: Rostos, Patel, Chen & Tufail, 2010; Rothenbuhler et al, 2009; Kang & Roh, 2009). Clinical trial reporting should also include change in visual acuity in the stability phase.

Recently Ross and colleagues have questioned the use of the full cohort VA measurement as an appropriate VA outcome. Following a large retrospective, real world cohort that subdivided patients into starting VA categories, it was shown that those with worse baseline vision gained most vision, beyond the 'ceiling effect' (Ross et al, 2013).

So far these discussions have demonstrated that although there are advantages of using VA as an outcome measure for nvAMD treatment studies there are also significant difficulties especially for individual point-to-point measurement. A further point of discussion is how well visual acuity represents visual function.

Mean distance VA outcomes do appear to broadly reflect what many patients informally communicate in the early part of their disease journey, that initially there is an improvement in vision and thereafter stability is reached. Once in the stability phase however, patients often do not report a change in vision when there is variation in point to point measured VA. This leads to discussion as to whether this measured change in VA is a true reflection of change in vision or visual function. Certainly there is good evidence that VA loss is associated with loss of day to day function: Scott reported on a cohort of 86 consecutive patients at the Wilmer Institute, Baltimore, of which 51 had normal visual acuity, and demonstrated that 2 quality of life questionnaires, the vision-specific Sickness Impact Profile and the Community Disability Scale did independently predict visual acuity (Scott et al, 1994). Similarly, a study of randomly selected participants in Maryland USA reported functional loss correlated with VA loss of 6/12 or more (West et al, 1997). This loss of function is also observed when macular degeneration is the cause: Mangione reported on 201 participants with AMD and noted that AMD did reduce quality of life based upon the Activities of Daily Living scale, however concluded that the clinical grading of the AMD did not explain the significant variation in visual function (Mangione et al, 1999). Hazel studied 28 participants with acquired macular disorders and concluded that although high contrast tests such as conventional distance VA measurement did correlate with performance loss as measured by vision related quality of life questionnaire, low contrast tests and reading speed better correlated with self-reported problems and visual concerns (Hazel, Petre, Armstrong, Benson, Frost, 2000). This finding was supported by McClure, who demonstrated in 100 patients with AMD that a combination of

reading index and distance VA had the best correlation with 'Daily Living Task Dependent upon Vision' visual function index (McClure, Hart, Jackson, Stevenson & Chakravarthy, 2000). Adding weight to this argument, Bansback reports a better correlation of quality of life utility with contrast sensitivity than distance visual acuity in 209 participants with either unilateral or bilateral AMD (Bansback, et al 2007). In the same cohort as he used to study the coefficient of variability of VA measurement, Patel also demonstrated that there was greater gain in contrast sensitivity using a Pelli-Robson Chart than ETDRS VA in the bevacizumab treated arm (Patel, Chen, Da Cruz, Rubin & Tufail, 2011).

So there is good documentation that VA alone can underestimate visual function. Frisen provides a good neuro-retinal explanation for this in a study of micropsia (perception of images to be smaller than they are) in macular oedema. Using quantitative assessment of micropsia as a sensitive indicator of photoreceptor displacement, it was estimated that only 44% of the normal neuro-retinal channels were required to give 6/6 ('normal') VA (Frisen & Frisen, 1979).

2.7. Conclusion

This chapter set out to assess the value of VA as an assessment tool for the outcome of treatment of nvAMD. It aimed to do this as a part of a study assessing the impact of normalising the re-assessment intervals of a cohort of individuals in the process of a nvAMD clinical service redesign.

The study demonstrated that during the stability phase of treatment reducing re-assessment intervals from 8 to 4 weeks resulted in an improvement of VA and that more treatments were given.

Using VA as an end point has advantages; it is quick and gives a numerical score that is easily interpreted statistically. The drawbacks are that it is tempting to translate the letters scores into accurate clinical outcomes. It is

clear from the experience of study and the work of others (Patel et al, 2008; Aslam et al, 2014) that use of VA as a surrogate marker of visit-to-visit disease activity accurate enough to base retreatment decision upon is inappropriate. It is surprising that national clinical guidelines have recommended retreatment based upon a loss in measured visual acuity letters alone: the NICE retreatment guidance of a loss of 5 letters for treatment of nvAMD with ranibizumab following 3 initiation treatments. (NICE TA155). Visual acuity can be a very useful tool in estimating the outcome of treatment of nvAMD but needs to be selected appropriately for what it is intended to be a surrogate for. Assessing the mean response of VA in cohorts, where variation is balanced amongst the participants is a much more appropriate use of this outcome.

With the knowledge that measuring VA change underestimates the change in visual function and the ability to perform visually dependent tasks (Mangiona et al, 1999; Hazel et al, 2000; Bansback et al 2007: Patel et al, 2011), better ways of assessing outcomes are required. This thesis goes on to explore some objective and subjective ways of assessing outcomes in order to address this need.

Chapter 3. Visual acuity and Ocular Coherence Tomography as Outcome measures of anti-VEGF treatment of Pathological Myopia associated Neovascular Macular Degeneration

3.1 Introduction

The previous chapter studied the effect of shortening reassessment intervals in patients receiving ranibizumab therapy for AMD complicated by a choroidal neovascular membrane (CNV). Visual acuity alone was used as an outcome measure of success of treatment and the merits and disadvantages of which were discussed. This chapter investigates the used of ranibizumab therapy to treat CNV secondary to myopia, and furthermore uses both visual acuity and OCT as measurement tools.

3.2 Background

Myopia, defined as a refractive error of -0.5 Dioptres or more affects approximately 1 in 3 people (Wolfram et al, 2014). Myopia is only defined as pathological however, when the refractive error is greater than 6 Dioptres and is accompanied by characteristic clinical features giving rise to an increased risk of visual loss. Pathological myopia (PM) is a principle cause of blindness in developed countries affecting 2–4% of Caucasians and 9–21% of Asian populations (Montero et al, 2010; Soubrane, 2008; Chan, Ohji & Lai, 2005). Choroidal neovascularization is the most common cause for the irreversible central vision loss that occurs in patients with pathological myopia. The natural history defines that approximately 90% of patients with myopic CNV (mCNV) will have visual acuity at 6/60 or less within 5-10 years of developing the complications, that the prevalence in PM is 4-11% and that those 50 years or younger will be predominantly affected. (Yoshida, Ohno-Matsui & Yasuzumi, 2003). The chance of the fellow eye being affected is high with around a third being affected within 8 years (Ohno-Matsui, Yoshida & Futagami, 2003).

3.2.2 Treatments for Choroidal Neovascular Membrane associated Pathological Myopia

3.2.2.1 Laser based therapies

Thermal laser, typically administered with an argon laser, is no longer considered as a useful treatment in the majority of patients. Because the wavelength of the laser causes collateral damage to the neurosensory retina, retinal pigment epithelium and choroid, treatment of subfoveal lesions induces severe immediate visual loss. Treatment of juxtafoveal lesions (within 200 microns of the centre of the fovea) and extra-foveal lesions (greater than 200 microns) is complicated by a high recurrence rate and long-term expansion of the laser scar that can creep over many months and years to involve the fovea (Chan et al, 2005; Secretan, Kuhn, Soubrane & Coscas, 1997). Those lesions that are so extrafoveal that they are asymptomatic can often be observed and may do well without intervention.

Transpupillary thermotherapy (TTT) uses diode laser and is less destructive to surrounding healthy tissues because its emission wavelength is close to infrared on the electromagnetic spectrum. It has low absorption by xanthophyll (a key macular pigment) and haemoglobin, so minimising nerve fibre layer damage, and allowing treatment through obscuring haemorrhage (Berger, 1997). Initial studies using photocoagulation in age-related CNV showed it to be effective demonstrating angiographic closure of the CNV in 7 or 9 eyes 40 weeks following 1 or 2 treatments (Ulbig, McHugh & Hamilton, 1993). Specifically for PM associated CNV, it has been shown to stabilise vision in about 2/3 of 74 cases that were studied retrospectively, with only 8% improving vision and the rest losing at least 0.1 LogMAR units of VA (Nabawi & Shaarawi, 2001). Following further reports of safety concerns such as macular infarction and poor efficacy, the National Institute for Health and Clinical Excellence (NICE) have not recommended its use outside of special arrangements for research (Benner, Ahuja & Butler, 2002; NICE, 2004).

3.2.2.2 Verteporfin- photodynamic therapy

Photodynamic therapy (PDT), using verteporfin was the mainstay of treatment until injectable treatment started being used. As with the use of TTT, the majority of study of PDT has been in age-related CNV, with the results being extrapolated to PM associated disease. In nvAMD at best PDT slowed down the progression of visual loss. Data from a large multinational, randomized clinical trial demonstrated a significant benefit for PDT over sham therapy for the primary end point of prevention of loss of 8 or more ETDRS letters (considered to be clinically significant vision loss, 72% versus 44%; p<0.01), but this effect was lost after 2 years (64% versus 49% p=0.11, Blinder, Blumenkranz & Bressler, 2003). Hayashi, when comparing bevacizumab with PDT in a consecutive group of 75 patients for mCNV demonstrated a significantly better VA in the former group at 12 months (Hayashi et al, 2009). Similarly in 31 patients receiving either bevacizumab or PDT, the PDT group demonstrated a mean worsening of VA after 12 months with significantly worse vision at 24 months (Ikuno et al, 2010). The results of the RETAIN study, a large multinational prospective randomised trial comparing ranibizumab and PDT is awaited.

3.2.2.3 Anti- Vascular Endothelial Growth Factor therapies

Based on landmark trials (ANCHOR, MARINA, CATT, and ABC trials), and clinical experience of treating patients with CNV secondary to AMD, ophthalmologists began to use anti-VEGF agents such ranibizumab and bevacizumab in an 'off-label' manner to treat CNV secondary to PM (Brown et al, 2006; Rosenfeld et al, 2006; Chakravarthy et al, 2012; Tufail et al, 2010). Small case series showed promise for these anti-VEGF agents in being able to substantially outperform PDT. Although different designs and durations, they report gains in VA letters with ranibizumab therapy. Silva reports a mean improvement from 20/100 to 20/50 at 6 months in 26 eyes, 15 of which were treatment naïve. (Silva et al, 2008). At the Jules Gonin University Eye Hospital a case series of 14 eyes demonstrated a mean VA

imporvment of 0.48 log-MAR (Konstantinidis, Mantel, Pournaras, Zografos, Ambresin, 2009). Mones et al reported on 23 eyes treated with a mean of 1.52 ranibizumab injections over 12 months gaining a mean of 9.5 letters (Mones, Amselem, Serrano, Garcia & Hijano, 2009). Another report on 16 eyes showed a mean gain of 3 letters at 12 months (Lai, Chan, Liu, Lam, 2009). Reviews are supportive of anti-VEGF therapy for CNV secondary to PM (Cohen, 2009; Ng, Kwok & Chang, 2012).

3.3 The aims of this study

The aim of this study is to evaluate the treatment of CNV secondary to pathological myopia with ranibizumab and to assess visual acuity as a functional measure and OCT as an anatomical measure of outcome.

At the time of performing this study no randomised controlled trials comparing ranibizumab and the PDT existed. Although ideally randomized controlled trials were needed to confirm results many retina specialists perceived that a PDT arm would be clearly inferior. It was felt that a case series demonstrating an increase in visual acuity in the context of well-documented natural history studies (Silva et al, 2008; Konstantinidis et al, 2009: Mones et al, 2009, Lai, 2009) showing that untreated myopic CNV results in a mean loss of VA would be a robust study design.

3.4 Methods

Approval was granted from the relevant ethical bodies for this study and it was performed in accordance with the Declaration of Helsinki and Good Clinical Practice. It was registered at ClinicalTrials.gov, identifier: NCT01037348.

3.4.1 Patient selection

To be included in the study patients had to be aged 18 years or more and have active primary or recurrent CNV secondary to PM. The lesion could be either in a subfoveal or juxtafoveal location, but a best-corrected visual acuity (BCVA) score of 24–78 Early Treatment Diabetic Retinopathy Study (ETDRS) letters was required. PM was defined as having a spherical equivalent of –6 Dioptres or more (or an axial length of 21mm or more on A-scan if this was unavailable), with characteristic chorioretinal changes.

In order to see the effect of the medication on the single pathology of CNV secondary to PM, patients were excluded if they met any of the criteria outlined in Table 4.

Surgical intervention in the study eye within 2 months of the screening visit
Current or previous macular laser photocoagulation
Treatment with intravitreal steroids, verteporfin-PDT or anti-VEGF agents in the study eye
Prior treatment in the study eye with vitrectomy or transpupillary thermotherapy
Those with current use or likely need for systemic medications known to be toxic to the lens, retina or optic nerve e.g. ethambutol, desferoximine
Concurrent use of systemic anti-VEGF therapy or previous treatment with intravenously administered bevacizumab
Concurrent use of chronic non-steroidal anti-inflammatory drugs (NSAIDs) for more than 7 consecutive days
Systemic or topical ocular corticosteroids for ≥ 3 consecutive days within 6 months prior to baseline
CNV from causes other than pathological myopia

Table 4. Patients were excluded from enrolment to the PM treatment study if they met the above criteria.

At Visit 1 (Baseline) all criteria for inclusion and exclusion were confirmed.

3.4.2 Ranibizumab dosing regimen

All study participants received one initial intravitreal injection of ranibizumab at visit 2. A qualified ophthalmologist experienced in intravitreal procedures administered this. A dose of 0.5 mg in 0.05 mL ranibizumab in solution was chosen as this reflected the product labeling for treatment of AMD (Summary of Product Characteristics (SmPC), 2007) and that used the recent case-series mentioned above (Silva et al, 2008; Konstantinidis et al, 2009; Mones et al, 2009, Lai, 2009).

The need for re-treatment was assessed at each subsequent visit (visit numbers 3-13) and was determined by a flow chart used set out in Figure 6. This was an 'as needed' (*Pro Re Nata* or *prn*) regimen with re-treatment largely determined by BCVA, symptoms and spectral domain Optical Coherence Tomography. A reduction in BCVA, symptoms of increased blurred vision or increased metamorphopsia were used as surrogate markers of the activity of the CNV. Visits were no more frequent than every 28 days.

1. Is there evidence of sub retinal or intra retinal fluid on the OCT?

YES- Retreat

NO- Go to question 2.

2. Has the patient had a decrease in BCVA by 5 letters or more or experience increased blurring or metamorphopsia?

YES- Go to question 3.

NO- No treatment and monitor in 1 month

3. Is there leakage on Fundus fluorescein angiography?

YES- Retreat
NO- No treatment and monitor in 1 month

Figure 6. The algorithm used to determine retreatment in the PM treatment study.

3.4.3 Outcome measures

Best corrected visual acuity (BCVA) and OCT determined centre point thickness were the principle outcome measures for this study.

BCVA assessment required the patient to be refracted by a certified optometrist at each visit. Initial testing was done at a distance of 4 meters. All letters were counted until fewer than 3 letters were read on a line. This number was then added to 30 to give the total letter score. If 4 letters could not be read on the first line then the distance was reduced to 1 meter and the total letter score equaled the total number of letters read.

Spectral domain OCT was performed on both eyes at each study visit prior to study drug administration, and a central macular cube of 512 A scans and 128 B scans was acquired through dilated pupils. Central macular thickness (centre point thickness) was recorded.

3.4.4 Endpoints

3.4.4.1 Primary endpoint

The mean change in BCVA from baseline to month 12 was the primary endpoint of this study.

3.4.4.2 Secondary endpoints

The secondary endpoints were the evaluation of the mean change in central macular thickness from baseline to month 12, the total number of treatments, the change in proportions of eyes with retinal fluid, and the safety of intravitreal injections of ranibizumab.

As with all clinical trials, safety data were collected. With regards to the secondary endpoint only serious adverse events (SAEs) were analysed. Events which are life threatening, fatal or which result in persistent or significant disability including a congenital birth defect, or that require inpatient hospitalization constituted the definition of an SAE.

3.4.5 Statistical analysis

The Mones study, reporting on the follow up of 23 eyes treated with ranibizumab at 12 months helped determine the required sample size (Mones et al, 2009). To detect a difference in mean BCVA of 10 letters, a sample size of 58 was calculated to have 90% power to detect mean change of 10 letters from baseline to month 12. This assumed a standard deviation of differences of 23, using a paired t-test with a 0.05 two-sided significance level. An expectation of approximately 10% drop out was allowed for, requiring a total of 64 eyes of 64 patients to be enrolled.

Data from all participants were analysed as long as they received at least one study injection of ranibizumab and had at least one further assessment of BCVA using the last-observation-carried-forward and intent-to-treat principles. Likewise safety was assessed for all patients who received at least one application of study treatment and had at least one post-baseline safety assessment.

Descriptive statistics for absolute values and changes from baseline were reported for each endpoint. For the McNemar tests (used to analyse the

qualitative aspects of the OCT) the category 'Questionable' was included with the 'Definite' category. Missing values (N/A) were excluded.

Due to the infrequency of the disease, only 7 participants were expected to be recruited into the study locally. To enable the recruitment target to be met 12 sites across the UK participated in the study. Correlation coefficients were calculated between the local and UK cohorts. A two-tailed probability was calculated an $P <= 0.05$ was taken as statistical significance.

3.5 Results

3.5.1 Demographic and baseline characteristics

In total, 7 participants were recruited locally and 65 throughout the UK. 62 patients completed the study, 1 withdrew due to an unsatisfactory therapeutic effect, 1 was withdrawn due to a protocol violation and 1 was lost to follow up. None of these 3 participants were in the local cohort. Demographic data are presented in Table 5. Most participants were Caucasian (90.8%), female (70.8%) and aged younger than 65 years (76.9%).

Mean age, years (SD)	55.5 (14.97)
Age distribution, n (%)	
< 50 years	21 (32.3)
50 to < 65 years	29 (44.6)
65 to < 75 years	10 (15.4)
75 to < 85 years	3 (4.6)
> 85 years	2 (3.1)
Gender, n (%)	
Female	46 (70.8)
Male	19 (29.2)
Predominant race n (%)	
Caucasian	59 (90.8)
Other	6 (9.2)

Table 5. Participant demographic data in the PM treatment study.

3.5.2 Primary endpoint

The mean BCVA increase for the local cohort of 7 participants was +16.5 letters (SD = 11.2, p <0.01, Paired T-test) over 12 months (Table 6 and Figure 7). The greatest improvement was seen following the initial treatment (+14.6 letters).

Visit Number	Mean VA (SD, ETDRS letters)	Mean CRT (SD, μm)
1 (Baseline)	55.7 (12.2)	381 (113.6)
2(Mandatory treatment)	70.3 (13.6)	282 (33.3)
3	71.2 (10.1)	273 (30.6)
4	69.1 (10.5)	260 (35.5)
5	74.6 (12.0)	261 (36.9)
6	74.4 (10.5)	261 (29.8)
7	74.0 (11.6)	255 (21.8)
8	73.3 (8.9)	243 (37.7)
9	69.9 (14.3)	272 (74.4)
10	70.0 (9.7)	271 (71.4)
11	74.4 (9.8)	260 (56.7)
12	70.4 (12.7)	268 (95.2)
13	74.9 (10.2)	269 (96.3)

Table 6. The mean (standard deviation) BCVA (ETDRS letters) and CRT (micrometres) for the local cohort of 7 participants in the PM treatment study. Figures are given for assessments at baseline, the mandatory treatment and subsequent as required visits.
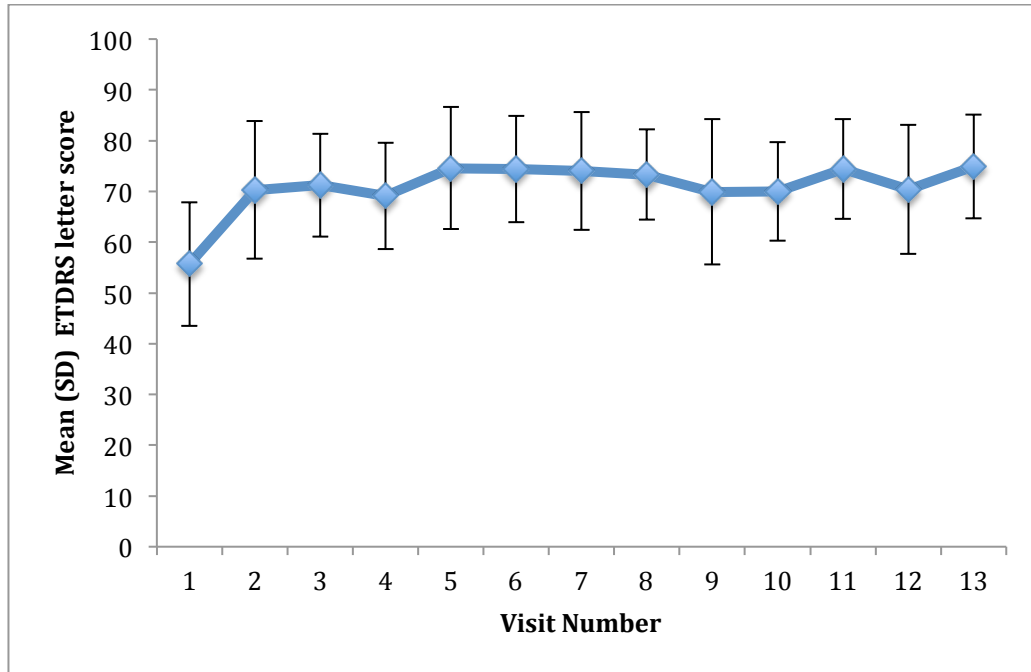
Figure 7. The mean (standard deviation) visual acuity for the local cohort of 7 participants in the PM treatment study. All 7 participants contributed to the mean at each visit. Visit number is displayed on the X-axis and absolute ETDRS BCVA on the Y-axis.

Analysing the whole UK cohort of patients, the mean VA improvement from baseline was 13.8 (SD=14.0, $p < 0.001$, Paired T-test Figure 8). The greatest improvement was observed in the first month of treatment (mean change, 8.7 letters $p < 0.001$); this was increased slightly throughout the 12-month period.

Visit number	Mean VA (SD, ETDRS letters)	Mean CRT (SD, μm)
1 (Baseline)	59.5 (13.6)	384.7 (130.9)
2(Mandatory treatment)	68.5 (13.6)	280.6 (89.7)
3	70.3 (13.0)	277.1 (87.2)
4	69.3 (13.2)	269.3 (80.6)
5	71.0 (13.7)	252.3 (77.3)
6	69.6 (14.3)	257.2 (76.5)
7	70.7 (15.5)	256.7 (82.7)
8	71.4 (14.4)	262.2 (87.7)
9	72.2 (13.9)	253.6 (83.8)
10	72.8 (14.4)	256.5(85.2)
11	73.5 (13.3)	249.8 (79.5)
12	72.6 (14.8)	248.7 (80.8)
13	73.0 (13.3)	251.4 (78.1)

Table 7. The mean (standard deviation) BCVA (ETDRS letters) and CRT (micrometres) for the UK cohort of 62 participants in the PM treatment study. Figures are given for assessments at baseline, the mandatory treatment and subsequent as required visits.
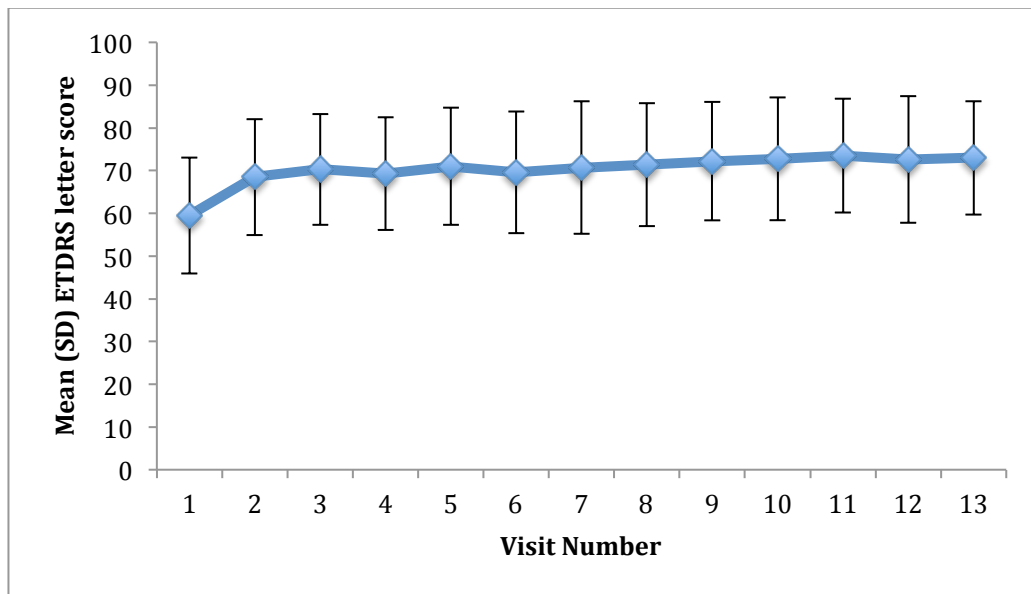
Figure 8. The mean (standard deviation) visual acuity for the UK cohort of 62 participants in the PM treatment study. Visit number is displayed on the X-axis and absolute ETDRS BCVA on the Y-axis.

3.5.3 Secondary endpoints

The local cohort of participants demonstrated a mean reduction in CRT of 116 (SD=72, $p<0.001$, Paired T-test) microns over the 12-month study period with the greatest reduction of 99 microns being observed after the initial treatment (Table 3 and Figure 4). The SD increases from visit 9 onward due to a single outlying result.
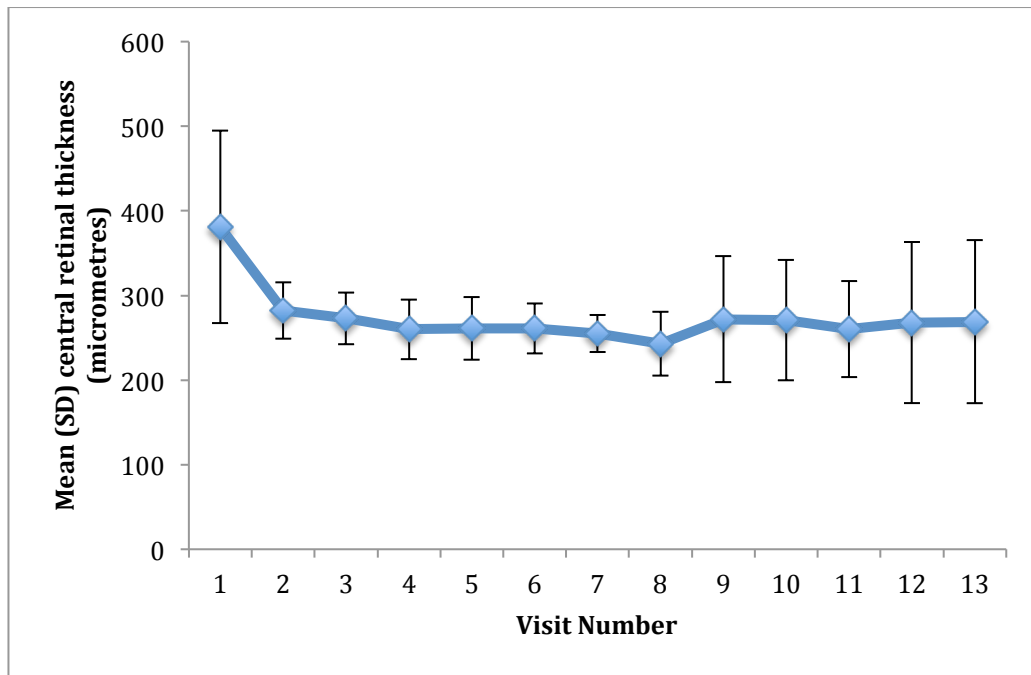
Figure 9. The local cohort mean (standard deviation) central retinal thickness is demonstrated for the PM treatment study. Visit number on the X-axis and mean change in central retinal thickness (micrometres) on the Y-axis.

For the UK wide cohort the mean change in central retinal thickness reduced by 135 μm (SD=109μm, p<0.001, Paired T-test) from baseline at 12 months (Figure 9). A substantial improvement of 104 μm (SD=90μm, p<0.001, Paired T-test) from baseline was observed at 1 month following treatment.
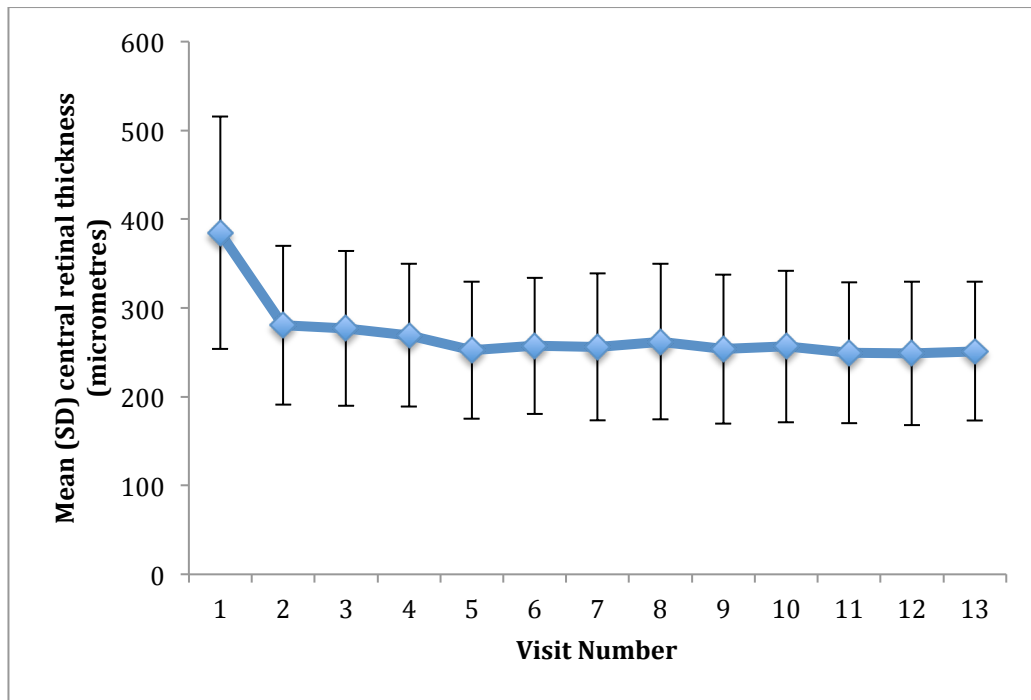
Figure 10. The UK cohort mean (standard deviation) central retinal thickness is demonstrated for the PM treatment study. Visit number on the X-axis and mean change in central retinal thickness (micrometres) on the Y-axis.

For the UK cohort, participants received a mean number of 3.6 treatments and thus a mean of 2.6 re-treatments was required after the prescribed first treatment. The median number was 3. The corresponding figures for the local cohort are 3.3, 2.3 and 2 treatments.

Qualitative analysis of the OCT images demonstrated that following treatment with intravitreal ranibizumab, as a proportion of eyes, subretinal fluid decreased from 67.7% to 7.7% ($p < 0.001$, Paired T-test), intra-retinal cysts decreased from 52.3% to 13.8% ($p < 0.001$, Paired T-test) and diffuse retinal oedema (DRE) decreased from 87.7% to 7.7% ($p < 0.001$, Paired T-test).

Three SAEs were reported in the UK wide series. There was one ocular SAE, a case of culture negative endophthalmitis (considered to be related to the

injection technique), which occurred in the local cohort. In addition, there were three non-ocular SAEs. There were two hospitalizations, one because of joint dislocation and the other because of pre-existing depression and anxiety, the latter occurring in a patient in the local cohort and was a pre-existing condition. A termination of a partner's pregnancy for social reasons was the third SAE.

Post hoc analysis of change in BCVA demonstrated that all but one patient avoided a loss of 15 letters or more, 32 (50.8%) gained 10 letters or more and 24 (36.9%) gained 15 letters or more. At baseline, the treatment eye was the worse seeing eye in 73.8% and this reduced to 59.4% at month 12.

3.6 Discussion

3.6.1 The efficacy and safety of ranibizumab as a treatment for CNV associated with Pathological Myopia.

This local dataset of 7 participants showed an improvement in visual acuity and reduction in central retinal thickness. Because of the small sample size rendering it open to greater bias, most of the ensuing discussion will be based upon the larger national dataset.

Ranibizumab treatment for CNV secondary to PM in a 1 + *prn* schedule improved VA and reduced CRT at 12 months compared with baseline. These improvements in vision were achieved with a low number of re-treatments after the initial dose. Furthermore, very few SAEs were reported.

These data, which at the time of study were the largest cohort to be prospectively studied show an improvement in VA of 13.8 letters at 12 months with a mean of 3.6 injections. Results from smaller case-series of ranibizumab therapy in CNV secondary to PM also consistently demonstrate significant improvements in VA with an equivalent improvement of 9-19

ETDRS letters (Silva et al, 2008; Konstantinidis et al, 2009: Mones et al, 2009, Lai, 2009). Based upon VA and CRT, the results add to the suggestion of ranibizumab's superiority to verteporfin-PDT with the VIP study (in age-related disease) showing verteporfin-PDT produces a loss of 10 letters at 12 months (VIP study group, 2001). The awaited RADIANCE study results, reporting on a direct comparison in a randomised trial, will better inform us.

The three reported SAEs did not reveal any safety concerns; all but the case of culture negative endophthalmitis were thought not to be related to the medication or the injection procedure (Lucentis SmPC, 2007). These findings are consistent with the well characterized safety profile demonstrated by ranibizumab in this patient population in published smaller, less robustly designed case-series (Silva et al, 2008; Konstantinidis et al, 2009: Mones et al, 2009, Lai, 2009). Given the significantly increased likelihood of retinal detachment in myopic eyes and eyes undergoing interventional procedures it was reassuring to observe that there were no reports of retinal detachment reported either in our study or the aforementioned studies. A much larger cohort of patients studied for a longer duration would be needed to more fully evaluate potential rare side effects.

The single-armed uncontrolled nature of this study limits its interpretation. The decision to have a singled armed open label design was taken as it was thought difficult to justify the allocation of patients to verteporfin-PDT as what appears to be, from the above discussion, a clearly inferior a comparator arm.

Although data from a multicentre study reduces the influence of single site bias the lack of a central reading centre leaves the interpretation of FFA and OCT images dependent upon the investigator. This may however, give a closer reflection of real world practice.

3.6.2 Visual acuity and OCT as outcome measures of the ranibizumab in treatment of CNV secondary to pathological myopia

This study confirms the notion that mCNV occurs predominantly in the work age group, and often in those age 50 years or younger (Chan et al, 2005). It therefore has the potential to have a significant economic impact by limiting the ability to perform visually dependent tasks, and so on career progression, even forcing early retirement (Soubrane, 2008). The question has to be asked whether high contrast VA or CRT as measured by OCT are the correct outcome measurements or a good proxy for visually dependent tasks?

3.6.2.1 Visual acuity

The arguments for and against using high contrast VA in a study setting have been made in Chapter 2 and concluded that measuring VA change often underestimates the change in visual function and ability to perform visually dependent tasks (Mangiona et al, 1999; Hazel et al, 2000; Bansback et al 2007; Patel et al, 2011). An additional point of discussion brought out by this study is which measurement of the change in VA is the most appropriate? This study used the mean change in VA from baseline to month 12. That is the mean of the entire visits post baseline out to month 12, and is an endpoint used in other studies (Ross et al, 2013). Another alternative would have been to use the change in mean VA from baseline to month 12 as in the ANCHOR and MARINA studies (Brown et al, 2006; Rosenfeld et al, 2006). The former has the advantage in that it minimises the impact of a non-representative result if the month 12 value is an outlier compared with the previous results as it would be expected to be in the stability phase of treatment (Tschour et al, 2013). The latter method has the advantage in that the slower increase in VA in the initiation phase of treatment does not dilute the true gain at 12 months (Tschour et al, 2013). As long as is appreciated that these two endpoints are different and either can be used.

3.6.2.2 Ocular Coherence Tomography

The advantages of the use of OCT to assess the outcome of treatment of mCNV and nvMD in general are numerous. In a compliant individual an OCT image, even with high resolution, typically takes a few minutes to acquire. An automated calculation of central retinal thickness produces a numerical value enabling the benefits of statistical analysis. Repeatability and reproducibility of spectral domain OCT central thickness is high, Giana reporting a highly reproducible central retinal thickness even of -6 to +6 microns using Bland-Altmann plots even when using different density of A-scan per B scan (Giana, Deiro & Staurenghi, 2012). The interpretation of OCT images is subject to inter-observer variability however. Patel reports on the study of 278 lines of 73 scan in patients with nvAMD prior to treatment. Two observers graded the images for the presence of intra-retinal cysts, sub-retinal fluid, diffuse retinal oedema, sub-retinal tissue and pigment epithelial detachment with agreement only ranging from 77-91% (Patel et al, 2009). Joeres, in a study of 60 images in AMD patients interpreted separately by 2 independent observers, demonstrated, however, that with training grading can be highly reproducible with only grading of sub retinal tissue, advanced CNV or RPE in the presence of poor visibility giving rise to the significant discrepancies. (Joeres et al, 2007).

The use of OCT does have a number of limitation and unknowns. Although the technology is advancing year upon year to give better resolution, caution has to be given when using automated interpretations. Firstly, to give an example, the Stratus (Carl Zeiss Meditec) time domain OCT does not give the same central retinal thickness measurement as its more advanced spectral domain successor, the Cirrus (Carl Zeiss Meditec). Grover and colleagues demonstrated that mean centre-point thickness in the same 36 healthy eyes was 166.9 +/- 29µm using a Stratus machine but 225.1 +/- 17.1µm (p<0.0001) using the Cirrus (Grover, Murthy, Brar & Chalam, 2010). This knowledge means that technology needs standardising within trials and caution given when interpreting across trials. Secondly, when OCT data

from the ABC trial were being analysed it was remarked upon that the automated delineation of the retinal structures such as the internal limiting membrane and the RPE (the structures between which retinal thickness is calculated) were often misplaced and required manual placement (Keane et al, 2012). As there was no central reading centre to verify the images in our study, errors in this figure are likely.

There are a number of OCT acquired parameters that could be used to assess macular morphology. Our study chose to use centre point thickness as the CRT as myopic CNVs are often small. The term 'CRT' or central retinal thickness differs between studies so again caution needs to be given when interpreting this value. Many studies, including those in chapters 5 and 6 use the mean thickness of the central $1mm^2$ subfield thickness, taken as a disc from the point of fixation as the CRT. This is because age-related CNVs and so their sequelae of retained? fluid are often larger.

Qualitative analysis of OCT images is also helpful. Our study demonstrated the proportion of eyes with intra-retinal, sub retinal and DRE was reduced at 12 months compared with assessment prior to treatment. Keane et al correlated qualitative OCT features with VA in the ABC trial involving AMD patients and so gives justification for the use of change or the presence of these features as re-treatment criteria (Keane et al, 2012). In an analysis of the 121 treatment naïve patients randomised to receive either bevacizumab or standard of care, OCT determined reduction in diffuse retinal oedema correlated strongly with increase in VA (p =0.01). Surprisingly, as it is often used as re-treatment criteria in many clinical trials such as the CATT and IVAN studies (Martin et al, 2012; Chakravarthy et al, 2012) sub retinal fluid did not (p=0.932). A larger volume of sub-retinal tissue at baseline also strongly correlated with decreased VA at the 54-week study end point (p<0.001). Furthermore Schmit-Urfuth's group reported that the integrity of the External Limiting Membrane (representing the photoreceptor layer) is the most important spectral domain OCT biomarker correlating with VA at any point during the treatment of nvAMD with ranibizumab (Roberts et

al, 2014). Through the 24 months study of 20 treatment naïve patients the correlation was strong, but still did not serve as a predictive biomarker for visual prognosis prospectively.

Central retinal thickness, an objective anatomical measure is used in this study as proxy for functional outcomes. Results show that there is justification for this in that there is a strong correlation between mean increase in VA and mean decrease in CRT (p<0.001). Unfortunately data was not collected to enable a qualitative change in OCT parameters to be correlated. As discussed, VA is not sufficient to inform about the ability to perform visually dependent tasks. The following chapter studies the use of patient reported outcome measures and correlates them with VA and CRT.

3.7 Conclusion

The results of this study show that ranibizumab used in an 'as required' re-treatment manner is an effective therapy for mCNV. It confirms the improvement in VA seen in smaller retrospective case series and supersedes performance of laser-based therapies. Few SAEs were identified in line with other ranibizumab-based studies.

Central retinal thickness, as measured by OCT performed well with its decrease correlating well with improvement in VA. Quantitative changes thought to be markers of disease activity were used as re-treatment criteria. As there is a paucity of literature on the treatment of mCNV, extrapolation is often taken from experience with nvAMD. The results of this study are consistent with that found with AMD, with improvement in VA possibly out performing its age-related counterpart. There is still a lack of information about how well the change in the surrogate markers of VA and CRT represent visually dependent function. Nonetheless the National Institute for Health and Clinical Excellence recently approved its used in the NHS (NICE TA298, 2013).

Chapter 4. Patient Reported Outcomes as Outcome Measures of anti-VEGF treatment of Pathological Myopia associated Neovascular Macular Degeneration

4.1 Introduction

Chapter 3 reported on the use of Visual Acuity (VA) and Central Retinal Thickness (CRT) as outcomes measures of anti-Vascular Endothelial Growth Factor (VEGF) treatment of myopic Choroidal Neovascularisation (mCNV). It concluded that there are benefits with the use of both of these biomarkers, in particular their use in parallel being of synergistic value in validating findings. It also discussed their limitations especially that they may not represent visual function or quality of life.

It could be argued that the ultimate goal of any treatment of non-life threatening disease is to restore the effect of disease on an individual's quality of life. Although surrogate markers of disease activity are important in measuring immediate response to therapy, or indeed can help with retreatment decision making it is also prudent to measure an individual's perception of disease effect. The National Institute of Health and Clinical Excellence bases funding arrangements not just on efficacy of treatments but also cost effectiveness using quality of life health utilities. An example of this is the decision of the NHS to fund ranibizumab for nvAMD (NICE TA 155). Quality of life measurements aim to give a more holistic approach in assessing treatment response. These measurements should be reported by patients themselves, and in fact the use of the self-reported status of healthcare has been suggested by some as to be of greater importance than any other outcomes that are clinical, physiological or carer-given (Deshpande, Rajan, Sudeepthi & Nazir, 2011). Patient Reported Outcomes (PROs) as defined by the US Food and Drug Administration are any report of the status of a 'patient's health condition that comes directly from the patient (i.e. without interruption of the patient's response by the physician

or anyone else' US FDA, 2006). This chapter assesses the use of PROs as a measurement of response to anti-VEGF treatment for mCNV.

4.2 Background

Pathological Myopia (PM) is a common disease with a significant risk of visual loss due to CNV (Montero et al, 2010; Soubrane 2008; Chan et al, 2005; Yoshida et al, 2003). The study in Chapter 3, the second largest prospective cohort at the time, showed that intravitreal anti-VEGF treatment of mCNV improved and then stabilised vision in this situation (Tufail et al, 2013a; Tufail et al 2013b). Of the 62 eyes that completed the study the mean change in VA was 13.8 ETDRS letters at 12 months, requiring a mean of 3.6 ranibizumab injections. The RADIANCE study, comparing photodynamic therapy to ranibizumab in a randomised clinical trial has now reported and also describes a significant mean gain in VA of 13.8 and 14.4 letters at 12 months in the 2 ranibizumab only arms (Wolf et al, 2014).

There are a number of different Patient Reported Outcome Measures (PROMs) that can assess vision related outcomes, each of which have been validated. The National Eye Institute Visual Function Questionnaire (NEI VFQ) is an example of a very commonly used one. With the help of patients with visual impairment, NEI VFQ was developed as a 51-point scale that sampled different aspects of visual function (Mangione et al, 1998). It was later refined to a more convenient 25 point scale, the NEI VFQ-25, with correlations to its 51 point predecessor showing it was still valid (Mangione et al, 2001). The NEI VFQ has subsequently been used to provide insight into the quality of life changes following anti-VEGF treatment for nvAMD. For example, it was used in a 24-month open label study of ranibizumab in nvAMD where baseline vision was maintained at month 12 and then declined, despite a mean of 7.6 (SD 4.1) injections. On this occasion the NEI VFQ-25 score improved at 6 months (p=0.03) and was subsequently maintained out to month 24 (Rakic et al, 2013). The NEI VFQ-25 has also

been used to study quality of life in mCNV in the RADIANCE study and demonstrated greater improvements in vision related function in the ranibizumab treated group (Wolf et al, 2014).

The MacDQoL is another example and is a 22-stem questionnaire designed to assess the impact of age-related macular degeneration on quality of life of individuals with the AMD. It sampled 159 individuals at a single time point and after removing 4 questions from the original 26 (mainly due to redundancy) analyses demonstrated it had excellent internal consistency and reliability. Furthermore the results demonstrated that AMD has a significant impact on independence, leisure activities, the ability to deal with personal affairs and mobility (Mitchell et al, 2005). It was further evaluated in a longitudinal study in 135 individuals with AMD and demonstrated excellent test re-test reliability over 12 months (Mitchell et al, 2007).

The domains of well-being and treatment satisfaction have not been studied in the context of treatment of mCNV and so this chapter focuses on two tools to assess these aspects of care.

4.2.1 The W-BQ12 Well-being questionnaire

The 12-item well-being questionnaire (W-BQ12, Riazi, Bradley, Barendse & Ishii 2006) was developed from the longer 22-item version and comprises 3 areas of assessment: Energy, positive well-being and negative well-being (Bradley & Lewis, 1990; Appendix A). Each of these 3 areas has a score of 12 points, making a total of 36 possible; a higher score indicates a better well-being. Although the PROM was developed for use in patients with diabetes, it has been shown to be a useful and reliable tool in patients with macular disease. Mitchell and Bradley analysed results from a self-completed survey of 1421 members of the then called Macular Disease Society and demonstrated consistency with its use in those with diabetes and worse scores in those registered as partially sighted or blind compared with those who were not. They concluded that 'The W-BQ12 will be useful in measuring

outcomes in rehabilitative and medical interventions and in researching factors affecting adjustment to MD' (Mitchell & Bradley, 2001).

4.2.2 The MacTSQ Treatment satisfaction questionnaire

The treatment satisfaction questionnaire MacTSQ (Mitchell, Brose & Bradley, 2007) was designed to assess treatment satisfaction of macular disorders using scales separated into two broad areas: impact of treatment, and provision of information and convenience. There are 12 questions in each of these 2 areas, with the maximum of 36 points in each area giving a potential overall maximum score of 72 points (Appendix B). A higher score indicates a better satisfaction. In a similar manner to the W-BQ12, the MacTSQ questionnaire was based upon a retinopathy treatment satisfaction questionnaire (Woodcock et al, 2005). The MacTSQ has been used to assess the outcome of treatment of AMD with anti-VEGF therapy in the IVAN trial, however the results are still awaited (Chakravarthy et al, 2013). It has not been used to study response in those with mCNV so far.

4.3 Methods

This study used the same participants as described in Chapter 3. In short, the aim was to recruit 65 participants with mCNV and treat them with a single administration of ranibizumab followed by further treatments on a monthly, as required basis out to 12 months. This was a single armed, open label design.

Both the W-BQ12 and the MacTSQ were completed at months 1, 6 and 12 with the W-BQ12 also being completed at baseline prior to treatment. It was given to the participants to complete independently but if required help from a research nurse was allowed. The questionnaire was administered at the study visits before the patients had ocular examination or treatment.

4.3.1 Statistical analysis

A full analysis set approach (all patients that had at least one treatment and one assessment following baseline) was used to analyse the full data set. The local cohort was not analysed separately due to the analysis of such a small data set being invalid (Riazi et al, 2006; Mitchell et al 2007). Missing data was handled by using a last observation carried forward method. Mean differences over time were tested using analysis of covariance. Mean change from baseline to month 12 in general, positive and negative well-being as well as energy was pre-planned. Correlations (Pearson product-moment correlation co-efficient) were used to study the relationship between VA, W-BQ12 general well-being and MacTSQ overall score.. For the W-BQ12, analyses was performed to compare participants when the treated eye being the better seeing eye (BSE) with participants when the treated eye was the worst seeing eye (WSE). A better seeing eye was defined as seeing 5 or more ETDRS letters better than the worse at baseline. The W-BQ12 was also sub analysed by groups dependent upon change from baseline VA (<0, 0-4, 5-9 and 10 or more ETDRS letters gained). For the MacTSQ, sub analyses compared the BSE versus WSE and groups depending upon the number of treatments given in 12 months (1, 2-3, or more than 3). Because these were exploratory end points, results were reported as descriptive only. P values were calculated using a paired t-test and a value of less than or equal to 0.05 was considered statistically significant.

4.4 Results

The VA and CRT results are described in detail in chapter 3, but to summarise in the cohort of 62 participants there was a mean VA improvement of 13.8 letters (SD=14.0, $p<0.001$), and a mean reduction in central retinal thickness of 135µm (SD=109µm, $p<0.001$) at 12 months (Tufail et al 2013a, Tufail et al 2013b).

4.4.1 W-BQ12 Well-being questionnaire

The mean general well-being score was 25.6 at baseline. Table 8 shows the change in general and sub-scales of well-being over time indicating a numerical increase in general well-being, positive well-being and energy, with a fall in negative well-being.

	Mean (SD) general well-being.	Mean (SD) positive well-being.	Mean (SD) negative well-being.	Mean (SD) energy.
Baseline (n = 65)	25.6 (6.96)	8.3 (2.74)	2.3 (2.80)	7.6 (2.54)
Month 1 (n = 59)	26.8 (5.03)	8.6 (2.59)	1.4 (1.95)	7.6 (2.30)
Month 6 (n = 61)	27.2 (6.08)	8.9 (2.61)	1.7 (2.47)	8.0 (2.48)
Month 12 (n = 61)	27.3 (6.35)	8.7 (2.66)	1.5 (2.60)	8.1 (2.44)
Change from baseline to month 12, p value	0.03	0.150	0.053	0.102

Table 8. The baseline and time point scores of the general well-being and subscales.

The change in general well-being from baseline to month 12 was significant (25.6 to 27.3, p=0.03) but the change in the subscales did not reach statistical significance.

When analysing patients who had treatment to their BSE, the change in general well-being, energy, positive well-being and negative well-being at 12 months were +2.1, +0.4, +0.2 and -1.5. When patients who had treatment to

their WSE was studied the changes were +1.7, +0.5, +0.8 and -0.5 respectively. Data for the general well-being is shown in Table 9.

	Mean W-BQ12 score BSE (n=15)	Mean W-BQ12 score WSE (n=44)
Baseline	23.2	27.2
Month 1	24.8	28.0
Month 6	25.9	28.2
Month 12	25.3	28.9

Table 9. The baseline and time point general well-being scores depending on whether the treated eye was the better and worse seeing eye. Note that there were 6 eyes where the baseline was neither better nor worse than the fellow eye (within 5 ETDRS letters of each other) and so total analysis was performed on 59 participants.

At 12 months participants who had achieved an increase in VA had an increase in general well-being but those who lost VA demonstrated a worsening of well-being (Table 10). Note this was not the case at 6 months for those that had lost vision.

	Mean general well-being score (number)			
ETDRS letter change from baseline (number of participants)	<0 (8)	0-4 (16)	5-9 (10)	> 9 (28)
Baseline	23.4	25.6	24.6	26.6
Month 1	20.9	26.0	26.5	28.7
Month 6	26.1	25.4	28.0	27.5
Month 12 (change from baseline)	22.6 (-0.8)	29.1 (+3.5)	27.0 (+2.4)	28.7 (+2.1)

Table 10. General well-being score over the course of the study, sub-grouped by improvement from baseline VA.

4.4.2 MacTSQ Treatment satisfaction questionnaire

The mean treatment satisfaction at month 1 was 55. This increased significantly at 12 months to 64.9 (p<0.001) as did both impact of treatment, and information provision and convenience subscales (Table 11)

	Mean (SD) treatment satisfaction	Mean (SD) impact of treatment	Mean (SD) information provision and convenience
Month 1 (n = 62)	55 (17.88)	26.2 (7.87)	28.7 (10.85)
Month 6 (n = 59)	58.8 (16.21)	29.6 (7.31)	29.2 (9.73)
Month 12 (n = 61)	64.9 (9.23)	32.0 (4.88)	32.9 (6.03)
Change from baseline to month 12, p value	<0.001	<0.001	0.014

Table 11. The baseline and time point changes in overall treatment satisfaction and subscales scores.

There was a similar increase in MacTSQ for both the BSE and WSE at 12 months (55.4 to 65.4, and 54.8 to 64.4) but at 6 months only treating the WSE showed an improvement (54.8 to 60.0 at 6 months and 55.4 to 55.4 at 12 months). Patients receiving 2-3 injections had a numerically higher increase in MacTSQ (51.0 to 66.8) from month 1 to 12 compared with those receiving only 1 or >3 treatments (Table 12).

	Mean treatment satisfaction		
Number of Treatments (number)	1 (13)	2-3 (21)	3 or more (26)
Month 1	65.0	51.0	53.1
Month 6	66.5	58.4	56.8
Month 12 (change in score from baseline)	63.0 (-2.0)	66.8 (+15.8)	64.5 (+11.4)

Table 12. The MacTSQ score over the course of the study, sub-grouped by the number of treatments received.

4.4.3 Correlations between visual acuity, well-being and treatment satisfaction

Weak correlations were noted between VA and W-BQ12 general well-being, VA and MacTSQ total score and W-BQ12 and MacTSQ). (Table 13).

	r value at month 12 (p value)
VA and W-BQ12	0.02 (0.877)
VA and MacTSQ	0.02 (0.877)
W-B12 and MacTSQ	0.08 (0.537)

Table 13. The correlation co-efficients and two tailed probability tests for correlations between VA, W-BQ12 and MacTSQ at month 12.

4.5 Discussion

4.5.1 Patient reported outcome measurements in the treatment of myopic CNV with ranibizumab

This study showed that patients treated with ranibizumab for mCNV over a 12 month study period on an 'as required' basis had a small but statistically significant increase in overall well-being as measured by the W-QB12 scale. There is no benchmark W-BQ12 data for mCNV treatment, or indeed for nvAMD for comparison, but the increase in well-being observed with treatment would be in line with clinical expectation based upon a parallel improvement in visual acuity. The validity of the result is further supported by the 12-month observation that those who had achieved an increase in the VA had an increase in general well-being but those who lost VA demonstrated a worsening of well-being. Some caution has to be given to these conclusions as it is also noted that this was not the case in those losing vision at 6 months. The results show an improvement in energy and positive well-being as well as a fall in negative well-being, with only the later approaching significance. These changes in subscales are also in line with clinical expectation and therefore the explanation for their failure to reach statistical significance in likely to be that the study was underpowered.

Treatment satisfaction as measured by the MacTSQ improved throughout the study both in terms of the treatment burden, and information provision and convenience subscales. Like with the W-BQ12 instrument there is no benchmark data in the use of the MacTSQ for the treatment of mCNV or nvAMD and the results on the IVAN study are awaited where it was used as an endpoint for treatment of nvAMD. Intravitreal injections can often be associated with considerable anxiety, but an increase in the MacTSQ can be interpreted as habituation to this in the context of this particular treatment regimen and study environment. Both impact of treatment, and information and convenience improved. Caution has to be taken in assuming these findings would be extrapolated into clinical practice, as routine clinical

practice often does not involve the same degree of patient contact or indeed the same environment as clinical trial practice offers.

By the definition used in this study more individuals were treated in a WSE than in the BSE. Improvements were marginally greater in those treated in their BSE. This small difference along with the small number in the BSE group makes interpretation of this finding difficult, although it is tempting to conclude it is explained by an improvement in the BSE having a greater impact on overall visual function. The concept that health utility scores are associated with the VA and in particular VA with their BSE is well founded one however. Brown et al studied 80 patients with macular degeneration and associated visual acuity with their BSE, reporting that those with mild visual loss (20/20-20/25) were prepared to trade off 11% of their remaining life and those with severe visual loss (Count fingers to Light perception) 60% if normal vision could be restored (Brown, Sharma, Brown & Kistler, 2000). This findng does not appear to be unique to AMD and mirrored findings in 100 consecutive patients with diabetic retinopathy with respective values of 15% and 41% (Brown, Brown, Sharma & Shah, 1999). Using similar trade off utility values in a cohort of 325 vitreoretinal patients, Brown et al was able to find a closer correlation with VA in the BSE than the WSE (Brown, Brown, Sharma, Smith & Landy 2001).

There appears to be no easily identifiable explanation for the difference in month 1 MacTSQ results when subdivided by number of injections given during the study. It may be expected that the month 1 MacTSQ would be similar across the subgroups as at this time all participants would have received a single initiation treatment, however those receiving only 1 treatment was higher than in the groups recieivng more. An large imbalance in the numbers within each group may have distorted this result. Those participants having only 1 treatment had a fall in MacTSQ whereas those having 2-3 or more than 3 had an increase. This appears to be counterintuitive but could be explained by those not requiring treatment beyond the first injection percieiving that no further attempt is being made

to restore their vision and so being less content. No further subdivision on the subscales is valid here due to the low participant numbers but analyses from a larger scale trial, such as the anticipated IVAN study may be more informative (Chakravarthy et al, 2012).

4.5.2 The use of PROMS as an outcome measure

Following on from the discussion in previous chapters, it is rational to conclude that mean VA over a treatment period is a reasonable way to assess the benefit of treatment of neovascular macular degeneration. It has also been concluded that CRT is a reasonable surrogate marker for visual acuity. In this chapter no correlation has been identified between the change in VA and the change in both well-being and treatment satisfaction utilities studied. It is therefore not possible to conclude with a degree of certainty that the W-BQ12 and MacTSQ are valid PROMs tools in the assessment of treatment of mCNV with ranibizumab on an as required basis, and furthermore that VA is a marker of quality of life. It may that a study designed to specifically addressed this, with adequate powering may be able to conclude differently. The improvement in visual function as demonstrated by the NEI-VFQ tool in the RADIANCE study adds further weight to the suggestion of the validity of PROMS however. It has to be appreciated that although broadly coming under the umbrella of 'Quality of life' measurements these tools measure different concepts; the NEI-VFQ measures perception of visual function, the MacTSQ perception of treatment satisfaction and the W-BQ12 overall perception of well-being.

The advantage of using a PROM is that is offers a new dimension to assess an outcome of treatment. Outcomes can be broadly divided into clinical (efficacy or safety), humanistic (performance of role, emotional status) and economical (expenses or saving) and the use of PROMs is a move away from using traditional clinical biomarkers of the improvement in 'disease' such as VA alone (Deshpande et al, 2011). The principle disadvantage of the use of PROMS, apart from their often time consuming nature and dependency on

literative skills of the user, is that the questions posed are those of the study team. Although the development of the W-BQ12 and MacTSQ may have been informed by patients, ultimately the desgin was that at the discretion of the investigators (Riaz et al 2006, Mitchell et al 2007). A purer way of capturing perceptions of well-being may be to take a more qualitative approach; however validating and interpreting such methods can prove more difficult.

Overall, PROMs work synergistically with clinical markers reinforcing the use of all of these endpoints and as such they are becoming increasingly used to assess the outcome of treatments in a number of therapeutic areas. NHS England is currently using PROMs to assess the benefit and patient perceptions of orthopaedic and surgical procedures (NHS England, 2015). Although as of yet no areas of eye health care are subjected to such mandatory study this is likely to become an important marker of the quality of services in the future.

4.6 Conclusion

The study in this chapter was the first time that the W-BQ12 and MacTSQ tools were used to evaluate outcomes in the treatment of mCNV with as required ranibizumab. Results show that both well-being and treatment satisfaction improved over the 12 month study period and although subject to variability during the study and subscales being affected by low numbers, help to set a benchmark for their use. The study demonstrates that changes in PROMS are comparable to those in VA and CRT, and although provide a different dimension to measuring outcome, have the potential to work synergistically in evaluating such treatments.

Chapter 5. Change in retinal sensitivity following treatment of nvAMD using anti-VEGF therapy: using a lesion-guided microperimetry retinal sensitivity as an outcome measure

5.1 Introduction

The previous chapters have discussed the use of visual acuity (VA), central retinal thickness (CRT) and patient reported outcomes (PROs) as outcome measures in the treatment of neovascular macular degeneration. Conclusions were made that VA, although useful when assessing cohorts, is not good at assessing point-to-point change in vision. CRT, as measured by Optical Coherence Tomography (OCT) is a more useful tool for individual assessments between single time points but still qualitative analysis of each image is required to aid the assessment of treatment. PROs are a usual adjunct in assessing the overall impact of therapies for macular disease but specific utilities are required to assess specific aspects of treatment and its outcome. VA provides a functional assessment of vision and OCT provides a morphological assessment of the macula. The chapter assesses and evaluates the use of microperimetry (MP) in determining retinal sensitivity as a part of an objective and functional outcome of treatment of neovascular AMD.

5.2 Background

Age-related macular degeneration is the commonest cause of sight loss in the elderly population (Owen et al, 2012). The neovascular subtype, representing about a fifth of incident cases is treatable with anti-angiogenic intravitreal injections (Brown et al, 2006; Rosenfeld et al, 2006; Martin et al, 2011; Charavarthy et al, 2012, Schmidt-Erfurth et al, 2014). The majority of affected individuals require therapy in the long term in an attempt to maintain vision (Rofagha et al, 2013; Tufail et al, 2014; Airody et al, 2014) with the maximal restoration of visual function being the goal of treatment. Once maximum visual potential has been reached, most individuals receive

discontinuous therapy, being retreated either when there are signs of disease activity (reactive), or when activity is predicted to return (proactive) (Lalwani et al, 2009; Berg, Pederson, Sandvik & Bragadottir, 2015). As discussed in previous chapters these signs of disease activity, and so response to treatments, are commonly measured by distance visual acuity assessment using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart and by assessment of retinal morphology as measured by optical coherence tomography (OCT). Visual acuity, because of its large coefficient of variability between measurements (Patel et al, 2008; Aslam et al, 2014) can be considered better placed to assess the response of a cohort of individuals or the mean response of an individual over a series of observations rather than judge response between single time points. Central Retinal Thickness (CRT) and in particular qualitative aspects of retinal morphology, as measured by OCT has good reproducibility and so can be considered well placed to measure outcomes between single time points. Despite being able to use these two biomarkers of visual function synergistically to aid the overall assessment of response to treatment there is still disconnect between patients' perceptions of visual change and conventional assessment methods such as VA (Legge, Ross, Isenberg & LaMay, 1992) and CRT (Munk et al, 2013; Cho et al, 2013).

Perimetry is a psychophysical method of assessing retinal sensitivity whereby a stimulus of given size and intensity is presented in the field of vision corresponding to the area of the retina under examination. An area of reduction in retinal sensitivity, termed a relative scotoma, or an area of total defect, an absolute scotoma, can be defined. Microperimetry, which enables the determination of a map of retinal sensitivity in a much smaller field and has shown promise as an objective tool of measuring macular function following treatment of nvAMD with anti-angiogenic agents (Squirrel et al, 2010; Baseler et al, 2011; Munk et al, 2013; Cho et al 2013). Munk et al chose to observe the size of the absolute scotoma over time and found a trend towards a reduction in its size with treatment but this did not reach statistical significance (Munk et al, 2013). Cho et al and Parravano et al

however showed an increase in overall retinal sensitivity over time (Cho et al, 2013, Parravano et al, 2010). The common theme with each of these studies was that an area of the macula was predefined before that start of the study and then used for examination of each participant. This concept has some inherent weaknesses in particular that the size of the retinal lesion does not correspond exactly to the area of examination.

Fixation is known to be impaired in individuals with AMD, particularly in late disease such as CNV (Tarita-Nistor, Gonzalez, Markowitz & Steinback, 2008; Pearce, Sivaprasad, & Chong, 2011). In these circumstances fixation can be eccentric, can occupy one or more new preferential retinal locations (PRLs) or can be unstable (Fletcher & Schuchard, 1997; Pearce et al, 2011). Fixation stability or rather how unstable the fixation is, is known to correlate with day-to-day tasks such as reading (Ergun, Maar, Radner, Barbazetto, Schmidt-Erfurth & Stur, 2003; Crossland, Culham & Rubin. 2005) and so is potentially an important measure of visual function when considering response to treatments.

This chapter studies the change in retinal sensitivity following treatment of nvAMD using anti-VEGF therapy using a lesion-guided microperimetry technique to specifically study an area of interest. It also evaluates fixation stability as an exploratory outcome.

5.3 Methods

This research followed the tenets of the Declaration of Helsinki. Informed written consent was obtained from all participants following an explanation of the nature and possible consequences of the study. Experimental protocols were approved by the York Neuroimaging Research, Ethics and Governance Committee and the University of York Ethics Committee.

5.3.1 Patient population and treatment

Treatment naïve patients that fulfilled the National Institute for Health and Clinical Excellence (NICE) criteria for treatment of nvAMD (Snellen VA 6/12 or less, nvAMD with evidence of recent disease progression) were recruited into the study. All participants had previously been assessed with Fundus Fluorescein Angiography as a part of routine care within 2 weeks of recruitment. Fluorescein angiography had been performed using a Carl Zeiss NM-1 camera with 2mls of 5% fluorescein being injected. Images were captured using a standard macular protocol in the choroidal, arterial, venous and late phases out to 10 minutes post-injection. Participants were excluded if they were unable to give written consent.

All participants underwent assessment with of Best Corrected Visual Acuity (BCVA) vision, CRT and retinal sensitivity (RS) at baseline (visit 1). Treatment was subsequently commenced with the intravitreal anti-VEGF agent that was requested by the local clinical commissioning group at that time (ranibizumab 0.5mg in 0.05mls, or Aflibercept 2.0mg in 0.05mls) and received monthly initiation doses under a standard aseptic technique via the pars plana. BCVA, CRT and RS assessments were performed following the subsequent three initiation doses making a total of 4 sessions per individual. At the same time as RS was measured, fixation stability, the ability of the eye to look at the target when the participant has been instructed to do so, was measured as an exploratory outcome.

5.3.2 Best Corrected Visual Acuity assessment technique

Participants had BCVA measured wearing their most up to date pair of distance glasses using an ETDRS chart (Pelli-Robson, Precision-Vision) at 4 metres. Each eye was assessed separately. If participants were able to read at least 4 letters on the top line at 4 metres then they were encouraged to read on until they were unable to read 3 or more letters on a subsequent line. A score of 30 was then added to the total number of letters read

correctly (to compensate for the 4 metre testing distance) to give the total letter score used for the study. If participants were unable to read 4 letters or more on the top line of the chart at 4 metres, the chart was moved to 1 meter and the total letter score was then equal to the total number of letters correctly read with no score being required to be added to this number (Ferris et al, 1982).

5.3.3 Retinal Sensitivity assessment by Microperimetry

Microperimetry was performed with the MP-1 micro-perimeter (Nidek Advanced Vision Information System [NAVIS]; Nidek Technologies, Padua, Italy) and was sequenced after BCVA assessment and before any other in order not to affect the measured retinal sensitivity. Participants had their pupils dilated, wore a patch on their fellow eye and undertook the assessment in a darkened environment. The fixation mark presented was in the form of a cross 2° in diameter, which if necessary could be increased in size. The light stimulus was presented in a sequential random grid pattern and consisted of 76 test points. The stimulus intensity ranged from 0-20 dB (0 dB refers to the strongest signal intensity of 127 cd/m2) in 1-dB steps. The MP-1 proprietary software 'follow-up function' was used for every follow-up visit ensuring the same areas of the macula were examined on successive occasions.

5.3.4 Central Retinal thickness assessment with Optical coherence tomography

Following microperimetry assessment the 1mm central subfield retinal thickness (micrometres) was measured using a spectral domain OCT with a standard macular cube assessment (Carl Zeiss Meditec).

5.3.5 Identification of the Area of Interest

Signs of the CNV and its sequelae (intraretinal fluid, subretinal fluid, pigment epithelial detachment) were identified anatomically both on the OCT and on a 10 minute frame of the FFA. An area, encompassing any of the activity seen with either OCT or FFA, was subsequently manually mapped onto the baseline MP grid pattern to identify which of the 76 points sampled were affected. This 'lesion plus sequelae' area denoted the 'area of interest' and was kept consistent throughout the subsequent data analyses.

5.3.6 Fixation stability assessment

As a part of the retinal sensitivity program, the MP automatically assesses eye fixation (that is if the eye is actually looking at the fixation target when the participant has been instructed to) about 25 times per second. The MP-1 then calculates the number of fixation assessments that are made that fall within 2 degrees or 4 degrees of the intended fixation target. Fixation stability is then defined in accordance with a study by Fujii and associates as follows:

1. Stable fixation: More than 75% of the fixation points inside the 2-degree-diameter circle,
2. Relatively unstable fixation: More than 75% fixation points inside the 4-degree-diameter circle and less than 75% inside the 2-degree-diameter circle and,
3. Unstable fixation: Less than 75% inside the 4-degree- diameter circle. (Fujii, DeJuan, Humayun, Sunness, Chang T & Rossi 2003).

5.3.7 Statistical analysis

The number of points in the area of interest was identified. Within this area the number and percentage of points that increased retinal sensitivity by 2dB and 4dB were calculated. Given that the co-efficient of repeatability for

the MP-1 has been described as 1.45 in Squirrel et al's study and 4.12-4.37dB in Wu et al's study, both a 2dB change and a 4dB change in RS were was chosen to be analysed at each location in the Grid (Squirrel et al, 2010; Wu, Ayton, Guymer & Lu, 2013).

Pearson correlation coefficients were calculated between the treated eye VA or CRT and the number of points gaining 2 or 4dBs. A two-tailed probability was calculated and $p <= 0.05$ was taken as statistical significance.

Data from participants that withdrew consent were not analysed. Missing data was handled by excluding that session from the analysis and the number of participants in a particular analysis was displayed. Fixation stability data is an exploratory outcome and so it is not appropriate to perform any statistical analyses.

5.4 Results

Sixteen participants were recruited into the study, of which 6 withdrew consent during the study. Of the remaining 10 participants all attended for VA and CRT assessments. Participant 7 had BCVA measured by the Snellen technique rather than ETDRS on the third visit so these data were excluded. Seven participants had a baseline MP assessment and only 8 had one or more further MP assessments. Of these follow up assessments only 6 had the first, 8 the second and one the final follow up assessments. Because of these low numbers data has only been analysed from the 8 participants with two of more MP assessments during the period of baseline (visit 1) to second follow up (visit 3) and missing data within these session was handled as described in the statisical analysis section of the methods. Reasons for this low MP rate included participants declining the assessment, technical failure and absence of staff.

Mean participant age was 76 years (67-87) with equal male:female distribution. There were 6 right eyes and 2 left eyes studied. The baseline charaterisitics are outlined below in table 14.

Particip ant number	Baseline BCVA for the Treated Eye (letters)	Baseline BCVA for the Untreated Eye (letters)	Baseline CRT for the Treated Eye (μm)	Baseline CRT for the Untreated Eye (μm)	Number of points in the area of interest (treated eye only).
1	75	85	309	261	76
2	62	75	269	235	51
3	79	71	292	288	55
5	40	79	314	288	29
6	55	69	272	266	50
7	69	94	335	315	57
8	70	68	260	237	(26 at visit 2)
10	28	72	738	317	29

Table 14. The baseline (visit 1) characteristics of the 8 participants (BCVA= best corrected visual acuity in ETDRS letters, CRT= central $1mm^2$ subfield retinal thickness in micrometers, and number points in the area of interest. Note that participant 8 had no baseline microperimetry performed so the area of interest was mapped onto the retinal sensitivity map at visit 2.

5.4.1 Best Corrected Visual Acuity

The BCVA values for individual participants during the study (Table 15) and the means of the cohort (Table 16 and Figure 11) are presented below.

Particpant number	BCVA visit 2 for the Treated Eye (letters)	BCVA visit 2 for the Untreated Eye (letters)	BCVA visit 3 for the Treated Eye (letters)	BCVA visit 3 for the Untreated Eye (letters)
1	64	79	75	85
2	80	79	81	79
3	80	74	80	75
5	51	90	63	80
6	48	67	74	69
7	82	89		
8	77	75	78	73
10	48	80	47	83

Table 15. Treated and untreated eye visual acuity (ETDRS letters) for the 8 participants over the 2 follow up sessions. A blank cell represents missing data.

Mean (SD) BCVA Baseline for the Treated Eye (letters n=8)	Mean (SD) BCVA Baseline for the Untreated Eye (letters n=8)	Mean (SD) BCVA visit 2 for the Treated Eye (letters n=8)	Mean (SD) BCVA visit 2 for the Untreated Eye (letters n=7)	Mean (SD) BCVA visit 3 for the Treated Eye (letters, n=7)	Mean (SD) BCVA visit 3 for the Untreated Eye (letters n=8)
60 (18.3)	77 (8.3)	66 (15.7)	79 (7.6)	71 (12.2)	78 (5.7)

Table 16. Treated and untreated mean and standard deviation (SD) of best corrected visual acuity (ETDRS letters) for the 8 participants over the 3 sessions, visit 1 (baseline) to visit 3. The number of sessions analysed (n)

was 8 in all cases except for visit 3 when the figure was 7 due to missing data.
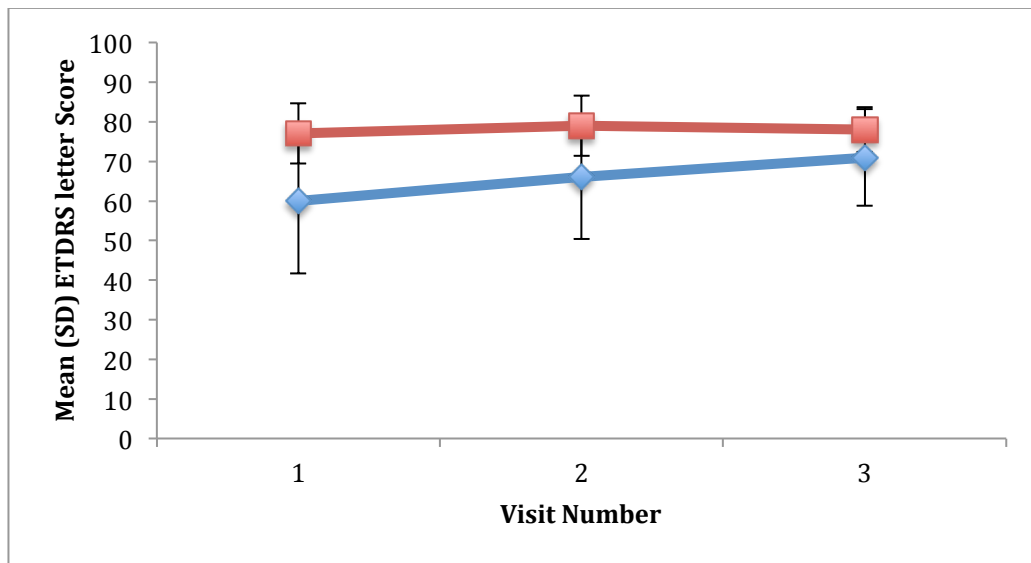
Figure 11. Mean (standard deviation) visual acuity (y-axis, ETDRS letter score) at visit 1 (baseline) to visit 3 (x-axis). Data from the treated eye is displayed in blue and data from the untreated eye in red.

5.4.2 Central retinal thickness

The CRT values for individual participants (Table 17) and the means of the cohort (Table 18 and Figure 12) are presented below.

Participant number	CRT visit 2 for the Treated Eye (µm)	CRT visit 2 for the Untreated Eye (µm)	CRT visit 3 for the Treated Eye (µm)	CRT visit 3 for the Untreated Eye (µm)
1	357	261	305	249
2	186	235	203	234
3	289	291	278	291
5	294	285	270	281
6	260	265	259	261
7	306	316	333	318
8	234	238	233	235
10	375	318	345	313

Table 17. Treated and untreated CRT (central 1mm^2 subfield retinal thickness, micrometres) for the 8 participants over the 2 follow up sessions.

Mean (SD) CRT Baseline for the Treated Eye (µm)	Mean (SD) CRT Baseline for the Untreated Eye (µm)	Mean (SD) CRT Visit 2 for the Treated Eye (µm)	Mean (SD) CRT Visit 2 for the Untreated Eye (µm)	Mean (SD) CRT Visit 3 for the Treated Eye (µm)	Mean (SD) CRT Visit 3 for the Untreated Eye (µm)
349 (159.4)	276 (31.7)	288 (61.9)	264 (50.9)	278 (48.3)	273 (33.1)

Table 18. Treated and untreated mean and standard deviation (SD) CRT (central 1mm^2 subfield retinal thickness, micrometres) for the 8 participants over the 3 sessions, visit 1 (baseline) to visit 3.
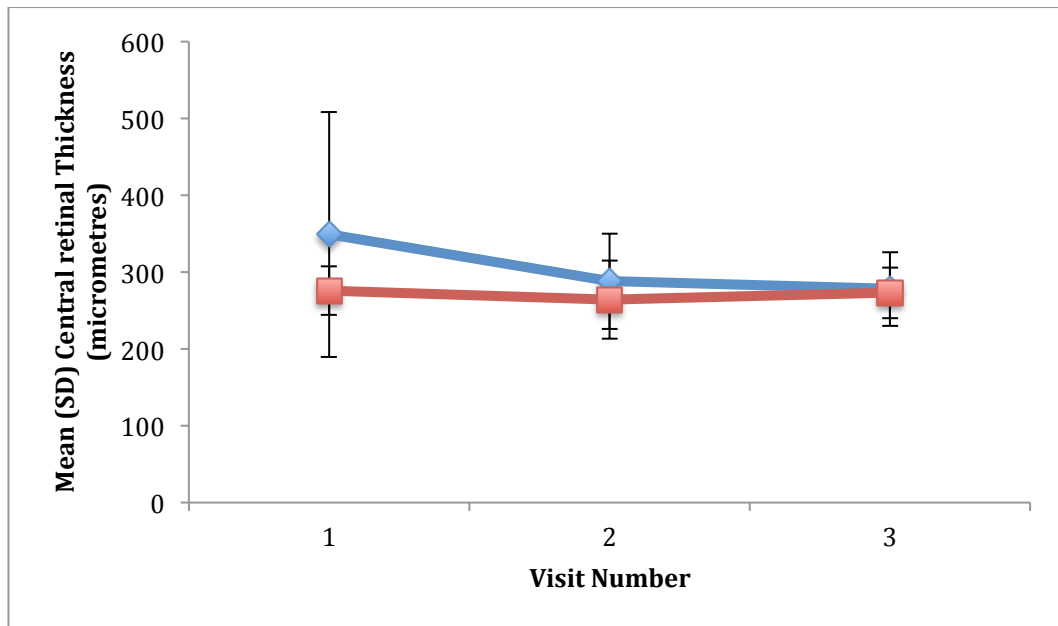
Figure 12. Mean (standard deviation) 1mm² central subfield retinal thickness (y-axis, micrometres) at visit 1 (baseline) to visit 3 (x-axis). Data from the treated eye is displayed in blue and data from the untreated eye in red.

5.4.3 Retinal sensitivity

The mean number of points in the area of interest was 47 (SD 17.3). The mean retinal sensitivity increased by 2.52dB (SD +/-1.2) at visit 3. The change in retinal sensitivity values as acquired by MP for individual participants (Table 19) and the means of the cohort (Table 20 and Figure 10) are presented below.

Participant number	Number of points in area of interest	Percentage (number) of points that increased by 2dB or more in area of interest at visit 2 compared with baseline (n=6)	Percentage (number) of points that increased by 4dB or more in area of interest at visit 2 compared with baseline (n=6)	Percentage (numbers) of points that increased by 2dB or more in in area of interest at visit 3 compared with baseline (n=7)	Percentage (number) of points that increased by 4dB or more in area of interest at visit 3 compared with baseline (n= 7)
1	76	14.5 (11)	11.8 (9)	44.7 (34)	22.4 (17)
2	51			29.4 (15)	19.6 (10)
3	55	63.0 (34)	24.1 (13)	78.7 (37)	42.5 (20)
5	29	27.6 (8)	13.8 (4)	58.6 (17)	34.5 (10)
6	50	44.0 (22)	20.0 (10)	62.0 (31)	46.0 (23)
7	57	33.3 (19)	28.1 (16)	77.2 (44)	54.4 (31)
8	26			73.1 (19)	38.5 (10)
10	29	13.8 (4)	13.8 (4)		

Table 19. Number of microperimetry points in the area of interest. Percentage and number of points that increased in retinal sensitivity by 2dB or more and 4dB or more for the 8 participants over the 2 follow up sessions. A blank cell represents missing data. n = the number of sessions analysed.

Mean percentage of points that increased by 2dB or more in acitve area at visit 2 compared with baseline (n=6)	Mean (SD) percentage of points that increased by 4dB or more in acitve area at visit 2 compared with baseline (n=6)	Mean (SD) percentage of points that increased by 2dB or more in acitve area at visit 3 compared with baseline (n=7)	Mean (SD) percentage of points that increased by 4dB or more in acitve area at visit 3 compared with baseline (n= 7)
33 (13.7)	18 (6.3)	62 (15.6)	39 (16.0)

Table 20. Mean percentage and standard deviation of points that increased retinal sensitivity by 2dB or more and 4dB or more for the 8 participants over the 2 follow up sessions. A blank cell represents missing data. n = the number of sessions analysed.
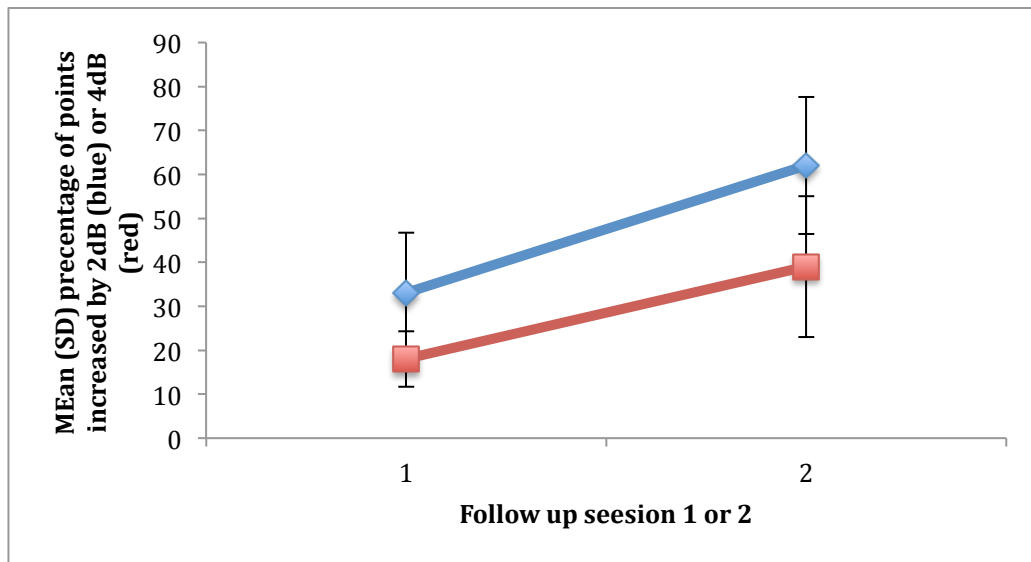
Figure 13. Mean (standard deviation) percentage of points that increased retinal sensitivity by 2dB or more (blue) and 4dB (Red) or more (y-axis) for the 8 participants over the 2 follow up sessions 1 and 2 (x-axis). The baseline (visit 1) data has not been displayed as it is defined as zero for all

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participants and change between baseline and first follow up may be affected by a significant learning effect (Wu et al, 2013).

5.4.4 Fixation stability

At baseline, 6 of 7 participants had relatively unstable fixation and 1 had stable fixation. Compared with baseline (visit 2 for subject 8 as baseline was missing), 2 subjects improved stability, 5 maintained stability and one got worse. In the case that fixation got worse data from visit 3 was missing (Table 21).

Participant number	Fixation at baseline	Fixation at visit 2	Fixation at visit 3	Change in fixation stability
1	R	R	S	Better
2	R		R	Same
3	R	S	R	Same
5	S	R	S	Same
6	R	R	R	Same
7	R	R	S	Better
8		S	S	Same
10	R	U		Worse

Table 21. fixation stability at baseline (visit 1), visit 2 and visit 3. S = stable fixation, R = relatively unstable fixation, U = unstable fixation (Fujii et al, 2003).

5.4.5 Correlation between VA, CRT and retinal sensitivity

Neither VA or CRT correlated even at a weak trend level with percentage retinal sensitivity change of 2dB or more or with 4dB or more (Table 22).

VA vs CRT (N=6)	VA vs 2dB (N=6)	VA vs 4dB (N=6)	CRT vs 2dB (N=6)	CRT vs 4dB (N=6)
-0.573 (P=0.234)	-0.323 (p=0.532)	0.030 (p=0.955)	0.607 (p=0.201)	0.582 (p<0.226)

Table 22. Correlations between visual acuity of the treated eye (VA), central 1mm² subfield retinal thickness of the treated eye (CRT) and percentage increase in retinal sensitivity of 2dB or more or 4dB or more (N= sample size).

5.6 Discussion

The mean increase in VA in this cohort of participants was 11 ETDRS letters following 2 treatments with anti-VEGF agents. This is much larger than the mean gain approximately 5.5 to 7.0 ETDRS letters in the corresponding time points of much larger published clinical trial data sets of (Brown et al, 2011; Chakravarthy et al, 2013; Schmidt-Erfurth et al 2014). The mean CRT reduction was 61 micrometers, which is smaller than expected from the same clinical trials (range approximately -120 to -180 μm). At first assessment it may appear that the study cohort of patients is therefore not representative of a typical population but caution has to be taken with this view, as the sample size is small.

5.6.1 The use of lesion-guided microperimetry retinal sensitivity as an outcome measure

The mean retinal sensitivity (RS) increased following the first and second treatments. This is in line with other published studies, although different methods of assessing RS have been used and so caution has to be taken when making judgment. Munk *et al* reported an increase in RS when mean central retinal sensitivity was studied in 64 treatment naïve patients treated with monthly ranibizumab over a 12 month period (Munk et al, 2013). On

that occasion, the microperimetry map covered 33 points over the central 12 degrees of the macula and showed the largest increase in RS 4 months post treatment and thereafter the RS plateaued. Correlation between the RS and VA was not reported upon but a decrease in absolute scotoma size over the course of the study was only significant at a trend level (p = 0.053).

Paravanno et al performed microperimetry on 5 occasions over 24 months during the study of 18 patients that were treated with ranibizumab. They were given an initial 3 monthly treatments then retreated with an 'as required' dosing regimen. The RS map consisted of 37 points over the central 12 degrees. On this occasion the VA improved up to 24 weeks then slowly declined whereas the mean RS continued to improve out to 24 months. In the second year the greatest disconnect between VA and RS was seen; the participants received a mean of 6.2 treatments achieving stable RS despite their VA slowly declining (Paravanno et al, 2010).

Cho et al used an OTI (Ophthalmic Technologies Inc.) microperimetry device to study 28 points in the central 12 degrees of the macula, in 42 eyes with newly presenting untreated nvAMD. Mean RS increased and the absolute scotoma decreased over the 12-month study period but no VA correlation data was reported.

The three aforementioned studies all use the mean change in RS of a large retinal map with a predetermined size. This technique has the advantage of capturing the full extent of a large area affected by nvAMD but also has the disadvantage of diluting the effect of any change with the surrounding healthy retinal tissue that would not be expected to change. This effect would be particularly strong in lesions of a smaller size. Baseler et al evaluated retinal sensitivity across 40 degrees but only analysed the central 10 degrees as this was deemed to be the area where damage was most likely to be (Baseler et al, 2011). In their study, in which a single case was reported upon, the scotoma decreased in size and the overall RS improved. Taking this concept of targeting the most appropriate area of the retina to

be studied a step further, the technique used in this chapter of the thesis studied the affected area of the macula alone. The location of the CNV and its sequelae was determined by late frame FFA and OCT morphology and so defined an 'area of interest'. Subsequently only RS change in this region was analysed.

Squirrel et al (2010) performed a study similar in duration to the one reported in this thesis whereby 10 eyes were analysed by MP out to one month following 3 ranibizumab injections. They reported a mean increase in RS of 2.9 dB (SD +/-1.5) over the 45 points measured within a central 12-degree map. Using repeatability measures it was determined that a 2dB change was likely to be significant change. Subsequently they reported that 9 of the 10 individuals had a change of at least 2 dB in 12 or more of the 45 points studied (Squirrell et al, 2010). This is a rather confusing statistic to report and even interpret, but the concept of how many points increase by 2dB is an interesting one. However, the down side of reporting on how *many* points change by a certain figure is that the size of the lesion and therefore number of points within the lesion (26-76 in this case) will differ between individuals. The study in this chapter therefore used the *percentage* of points within the area of interest that changed by 2dB and so attempts to correct for the size of the lesion. In fact the mean percentage of points that increased by 2dB or more in active area was 62% after 2 treatments. This seems an intuitively better way to represent the treatment effect.

Neither VA change nor CRT change came close to correlating with the increase in percentage of points achieving 2 or 4 dB improvement. Because of the low sample size it is therefore hard to interpret whether this result is due the study being underpowered or indeed whether there is in fact no correlation. Based upon these results it is again hard to validate RS used in this way as a useful tool to measure visual function for an individual response to treatment on a visit-to-visit basis.

Much as qualitative aspects of OCT morphology such as the presence of retinal fluid represent disease activity and so are used to guide treatment (Martin et al, 2011; Chakravarthy et al, 2012), future studies could be aimed to see if retreatment criteria could be based upon the signs of disease activity as represented by MP determined retinal function.

The weaknesses of this study have to be acknowledged, in particular the low numbers and short duration of follow up, when interpreting the data. Furthermore it has to be acknowledged that the participants may have undergone a significant learning effect between the first and second evaluations, as was noted by the work of Wu and colleagues (Wu et al, 2013). This potential bias was taken accounted for by not displaying the change between the baseline and first follow up examination in figure 5.3. Pre planning analyses on the correlation of VA, CRT and absolute RS may have provided useful information in being able to validate RS as a useful tool help guide re treatment decisions and should be considered for future studies.

Nonetheless this study demonstrated that there was improvement in RS within a high proportion of significant improvement points within the area of interest. These data would act as good pilot data for a larger study to confirm that this technique is a sensitive way of detecting RS change following therapeutic intervention for nvAMD. Demonstrating that RS can continue to improve or decline when VA and CRT plateau, particularly if individuals continue to notice a change in their visual function, adds to the argument for its routine evaluation in clinical practice. Alexander et al demonstrated that in 5 of 21 eyes in the 'stability phase' of their treatment as determined by stable VA and CRT when ranibizumab was not administered, RS decreased (Alexander, Mushtaq, Osmond & Amoaku, 2012). Retinal sensitivity as a functional outcome of MP could act synergistically along with VA and OCT to help determine outcomes or treatment response.

The main disadvantage of using MP as a routine assessment tool is that the time required to perform the examination can often be 8-12 minutes per eye. So to commit significant resources to do this its added value over and above OCT, which has an acquisition time of only around 1 minute per eye, would have to be certain. In addition, technical reliability and staff time need to be secured as both of these factors hampered data acquisition in this study.

5.6.2 The evaluation of fixation stability as on outcome measure

Of the eight participants, two improved stability, five maintained stability and one got worse. The general trend is toward maintenance or improvement although it has to be noted that 2 already had stable fixation at their initial assessment and so were subject to the ceiling effect. These observations are broadly in line with other studies in the literature that also used the Fujii method of assessing fixation on the MPI-1. Munk *et al* reports that 63% of eyes had relatively stable fixation, 27% unstable and 10% stable fixation before anti-VEGF treatment changing to 72%, 13% and 15% respectively at 12 months (Munk et al, 2013). The cross sectional study of 102 patients by Pearce et al, 76 of whom had ranibizumab treatment, demonstrated similar results in the treated group (Pearce et al, 2011).

Although the Fujii method of determining fixation stability is often used (Parravano et al, 2010; Pearce et al, 2011; Munk et al 2013) it is a relatively crude measure in that it is only a three-point scale (Fujii et al, 2003). Crossland reports a potentially more accurate way of assessing fixation stability by capturing fixation using a bivariate contour ellipse area (BCEA). This gives a numerical value that has been showed to better correlate with reading speed than the Fujii method (Crossland, Dunbar & Rubin, 2009). It was not possible to retrospectively capture raw numerical data necessary to calculate the BCEA or perform other analyses, but consideration should be given to using this method for future studies.

Just as discussion about the qualitative aspects of OCT and MP have suggested their usefulness in retreatment decisions, the same argument can be applied to fixation data. If it was clear that disease activity was threatening a PRL then a physician may be more likely to administer treatment.

5.6.7 Conclusion

The use of microperimetry to assess RS and fixation stability has merits in potentially being able to explain disconnect between visual function and conventional methods of assessing disease activity such as OCT and VA. Both quantitative and qualitative aspects need further study to evaluate if the principle downside of these additional measurements, that is the time taken to acquire the data, is outweighed by its benefits.

Chapter 6. Functional MRI Blood Oxygen Level Dependency response as an outcome measure of visual function following the treatment of neovascular Age-related Macular Degeneration: restoration of primary visual cortex activity.

6.1 Introduction

By performing specific studies the previous chapters have discussed the use of visual acuity (VA), central retinal thickness (CRT) and retinal sensitivity (RS) as outcome measures when assessing the affect of treating neovascular macular degeneration (nvMD). This chapter discusses the merits of using functional MRI as an outcome measure and compares it with these previously discussed methods.

6.2 Background

Treatment paradigms for neovascular Age-related, Macular Degeneration (nvAMD) have been discussed in detail in previous chapters. After the 'initiation phase' of treatment when monthly intravitreal injections are given, retreatment is in either a proactive or reactive manner (Brown et al, 2006; Rosenfeld et al, 2006; Martin et al, 2011; Charavarthy et al, 2012, Schmidt-Erfurth et al, 2014). Most patients can expect an initial gain in visual acuity followed by visual stability or a slow decline in the 'stability phase' (Rofagha et al, 2013; Tufail et al, 2014; Airody et al, 2014). Despite the routine use of VA measurement and OCT features these outcome measures do not give a complete representation of visual function or indeed the change in visual function in response to treatment.

Functional activity of the visual cortex as measured by the Blood Oxygen Level-Dependent (BOLD) response on MRI has the potential to be an objective measurement of visual response to retinal treatment and complement existing assessment modalities. As neuronal activity increases in the brain there is an increase in the amount of oxygenated blood

compared to deoxygenated blood. This is because neuronal activity causes a release of the neuro-transmitter glutamate, leading to the release of nitric oxide and ultimately dilation of blood vessels (Ogawa, Lee, Kay & Tank, 1990).

To enable the BOLD response to be a potential marker of visual function in those treated for nvAMD two principle assumptions need to be met. The first assumption is that there is the absence of significant re-modeling of the visual cortex in those with AMD so that retinotopic (retina to cortex spatial mapping) relationships are maintained. This assumption would mean that any BOLD change that is observed would be due to the treatment intervention and not natural recovery. A number of studies have debated this point. Baker et al reported on preliminary fMRI investigations into 2 patients with severe AMD and suggested that visual processing could be reorganised. It was demonstrated that peripheral retinal stimulation activated the area of the visual cortex only normally represented by foveal stimulation (Baker, Peli, Knouf & Kanwisher, 2005). Baker et al went on to qualify this statement, after replicating the study in 3 further individuals, by suggesting that this re-organisation may only occur if there was severe loss of foveal function (Baker, Dilks, Peli, & Kanwisher, 2008). To add to this argument Schumacher et al noted that this apparent degree of cortical plasticity could be related to eccentric viewing. (Schumacher et al, 2008). This debate about cortical reorganisation has swung the other way more recently when Baseler et al performed fMRI assessments of a much larger cohort of patients. All had established bilateral macular degeneration and no significant activity in the foveal representation was observed (Baseler et al, 2011). Studies in macaque monkeys with macular degeneration have confirmed this limited capacity for reorganisation in this so called lesion projection zone (that is the area of the cortex that represents the area of retinal defect) of the primary visual cortex (Smirnakis et al, 2005; Shao et al, 2013), but interestingly more extensive plasticity in the higher visual cortex zone V5 was noted (Shao et al, 2013). It is worth noting that this absence of

significant reorganisation occurs in the presence of both monocular and binocular retinal lesions, a point particularly important when studying nvAMD, which typically has a unilateral onset (Murkami, Komatsu, Kinoshita, 1997). So the balance of argument is now strongly in favour of concluding that there is no significant cortical re-organisation in long-standing retinal lesion acquired in adulthood.

The second assumption that needs to be fulfilled is that the visual cortex has the ability to recover function. Boucard et al performed an MRI study of the posterior pole anatomy in 9 AMD patients, average age of 72.6 years, and demonstrated a reduced grey matter volume compared with age-matched controls (Boucard et al, 2009). A similar anatomical study in adults with long standing retinal defects also showed grey and white matter volume changes (Noppeney, Friston, Ashburner, Frackowiak & Price, 2005). Although these studies seem to deny this second assumption they did not assess functional aspects of MRI and a direct relationship between structure and function may not be inevitable. To support the case that function can recover, Baseler et al has reported on a single case that showed clear improvement in the BOLD visual cortical response when nvAMD was treated with an anti-VEGF agent (Baseler, et al, 2011).

So the way seems to be paved to allow further assessment of the use of the BOLD response in measuring outcomes of retinal treatment. The aim of the study in this chapter was to determine the BOLD response of the visual cortex in a cohort of individuals with nvAMD before, during and after the initiation phase of treatment with anti-angiogenic therapy. Furthermore it aimed to determine how the BOLD response compares with the standard clinical methods of assessing vision and retinal anatomy.

6.3 Methods

This research followed the tenets of the Declaration of Helsinki. Informed written consent was obtained from all participants following an explanation of the nature and possible consequences of the study. Experimental protocols were approved by the York Neuroimaging Research, Ethics and Governance Committee and the University of York Ethics Committee.

6.3.1 Patient population and treatment

This study ran in parallel with the study described in Chapter 5 and on the same cohort of individuals. To recap, treatment naïve patients that fulfilled the National Institute for Health and Clinical Excellence (NICE) criteria for treatment of nvAMD (Snellen VA 6/12 or less, nvAMD, evidence of recent disease progression) were recruited in to the study. Participants were excluded if they were unable to give written consent. All participants underwent assessment with best-corrected visual acuity (BCVA) using an ETDRS chart, OCT (Cirrus, Carl Zeiss Meditec) and fMRI at baseline (visit 1). All participants were commenced on the recommended intravitreal anti-VEGF agent at that time (ranibizumab 0.5mg in 0.05mls, or Aflibercept 2.0mg in 0.05mls) and received 3 monthly initiation doses under a standard aseptic technique via the pars plana. Assessments (VA, OCT and MRI) were repeated following the three initiation doses making a total of 4 sessions (visit 2-4) per participant.

6.3.2 Visual acuity technique

This was as described in chapter 5 with participants having best corrected visual acuity (BCVA) measured using an ETDRS chart (Pelli-Robson, Precision-Vision) at 4 metres using their most up to date pair of distance glasses.

6.3.3 Ocular coherence tomography

This was also as described in chapter 5. Following pupillary dilation a standard macular cube assessment on a spectral domain OCT (Cirrus, Carl Zeiss-Meditec) allowed the 1 mm^2 central subfield central retinal thickness to be recorded.

6.3.4 Functional MRI technique

At each visit there were two assessments of the treated eye and two of the untreated (control) eye giving a total duration of examination of up to 30 minutes.

The stimulus was generated using MATLAB (Natick Laboratory; MATLAB and Statistics toolbox release, The Mathworks, Inc., Natick, Massachusetts, USA.) and presented using an LCD projector (EPSON GB5900, 60Hz refresh rate, maximum luminance = 6000 candelas per metre squared - Minolta LS110 photometer). A 45 degree-tilted, front-silvered mirror was placed in front of the subject's head, so the projection could occur onto the face from the light source. A diffusing acrylic film was fit to some clear acrylic goggles, which were placed over the subject's eyes. This film attenuated the mean luminance to 1350 cd/m^2. Additionally, underneath the goggles, either the left or right eye of the subject was occluded with a sterile fabric eye patch while measurements from the other eye were made. Participants were instructed to keep both eyes open and to remain still during the scans; no fixation was required. Foam padding was used around the participant's head to minimise movement, with earplugs provided to protect from the noise of the scanner. The stimulus was a contrast reversing Ganzfield, reversing at 6Hz. The stimulus was presented for 18 seconds and interleaved with periods of mean luminance (grey screen) of 18 seconds each. This 36 second stimulus cycle was repeated 8 times per scan.

Structural data were acquired using high-resolution T1-weighted images and inplane structural images. Functional MRI data were acquired using an eight-channel, phase-array head coil tuned to 127.4 MHz, on a General Electric Signa HD Excite 3T MRI scanner. Gradient recalled echo-pulse sequences were used to measure T2*-weighted blood oxygen level-dependent (BOLD) data. The imaging parameters used for the T2* weighting were TR = 3000ms, TE = 30ms, Flip angle = 90degrees, matrix size = 64 x 64, field of view = 192 mm, slice thickness = 3 mm and voxel size = 3 x 3 x 3mm^3 In additional to the functional data a whole head high resolution T1 weighted anatomical image was acquired with the following parameters: TR = 7.92ms, TE = 2.9ms, matrix size = 256 x 256 field of view = 290 mm, slice thickness = 1 mm and voxel size = 1 x 1.13 x 1.13mm^3 This high resolution brain image was used as a common space for comparison of data across sessions. To aide alignment of data across sessions an additional anatomical image was acquired as an intermediate step for aligning functional data to the high-resolution whole brain dataset. This proton density weighted image was acquired with the same slice prescription as the functional data and with the following parameters: TR = 2500ms, TE = 34.9ms, matrix size = 512 x 512 field of view = 192 mm, slice thickness = 3 mm and voxel size = 3 x 0.375 x 0.375mm^3 Pre-processing stages included; MCFLIRT motion correction (Jenkinson, Bannister, Brady & Smith, 2002) using FLIRT (FMRIB's Linear Registration Tool), slice-timing correction using Fourier-space time-series phase-shifting and linear detrend filtering, to filter out any inconsistencies. Functional time-series were high-pass filtered to remove baseline drifts.

Data analysis was performed primarily in MATLAB using the publicly available mrVista toolbox (http://white.stanford.edu/software). Data were averaged across scans for each participant and were aligned to the high-resolution anatomical volume and visualised in 3D.

Based on anatomical criteria two regions of interest (ROI's), 5 mm in diameter were chosen in each hemisphere from each participant. One was

chosen at the posterior occipital pole (PP), retinotopically representing the macula region, and the second in the mid-calcarine (MC) region representing a peripheral location in V1 to act as a control. The percent BOLD signal change was averaged across all voxels within a given ROI. The BOLD response (BR = a ratio) from the PP was controlled for the expected variation (Rosengarth et al, 2013) in intersession response by correcting it by the MC activity. To further minimise this variation the treated eye response was also controlled by the untreated eye and so the final BR was calculated using the following equation:

BR　　　　=　　　　(PP/MC) Treated / (PP/MC) Untreated

6.4.5 Statistical analysis

The stimulus driven amplitude of the signal recorded at each voxel within the ROI was computed via a vector mean calculation. Data was averaged across hemispheres within each scan session.

Pearson correlation coefficients were calculated between the treated eye VA, the treated eye CRT and the BOLD response. A two-tailed probability was calculated and $P <= 0.05$ was taken as statistical significance.

Data from participants that withdrew consent were not analysed. Missing data was handled by excluding that session from the analysis and the number of participants analysed displayed.

6.5 Results

Sixteen patients were recruited into the study, of which 6 withdrew consent during the study. Data from the remaining ten patients were analyzed with seven right and three left eyes affected. Mean age was 75.7 years (67-87) with equal male to female distribution. All patients attended for their treatment and VA/OCT assessments on schedule but only five attended all

four neuroimaging sessions. Of the remaining five, all attended for their first assessment and two further assessments with one not attending the second and two not attending their third or fourth assessments. Participant number seven had BCVA measured by the Snellen technique rather than ETDRS on the third visit so this data was excluded. The baseline VA, CRT and BOLD characteristics are outlined in Table 23.

Participant number	Baseline BCVA Treated (letters)	Baseline BCVA Untreated (letters)	Baseline CRT Treated (µm)	Baseline CRT Untreated (µm)	BOLD (PP/MC) Treated	BOLD (PP/MC) Untreated
1	75	85	309	261	0.282	0.591
2	62	75	269	235	1.462	1.453
3	79	71	292	288	0.430	0.459
4	60	84	366	245	0.132	0.473
5	40	79	314	288	0.187	0.388
6	55	69	272	266	1.991	1.183
7	69	94	335	315	0.824	1.190
8	70	68	260	237	0.337	1.166
9	62	74	233	258	0.373	0.479
10	28	72	738	317	0.439	-0.628

Table 23. The baseline (visit 1) characteristics of the 10 participants' treated and untreated eyes (BCVA= Best Corrected Visual Acuity in ETDRS letters, CRT= central 1mm^2 subfield retinal thickness in micrometers, BOLD = Vector mean projected amplitude of Blood Oxygen Dependency Level).

6.5.1 Visual acuity

The VA values for individual participants and the means of the cohort are presented below.

Participant number	BCVA visit 2 Treated (letters)	BCVA visit 2 Untreated (letters)	BCVA visit 3 Treated (letters)	BCVA visit 3 Untreated (letters)	BCVA visit 4 Treated (letters)	BCVA visit 4 Untreated (letters)
1	64	79	75	85	77	76
2	80	79	81	79	78	78
3	80	74	80	75	85	78
4	60	85	60	83	55	78
5	51	90	63	80	66	85
6	48	67	74	69	76	74
7	82	89			81	89
8	77	75	78	73	78	78
9	49	73	57	75	46	78
10	48	80	47	83	45	79

Table 24. Treated and untreated eye visual acuity (ETDRS letters) for the participants, 1-10 over the 3 follow up sessions. A blank cell represents missing data.

Mean (SD) BCVA Baseline Treated (letters n=10)	Mean (SD) BCVA Baseline Untreated (letters n=10)	Mean (SD) BCVA visit 2 Treated (letters n=10)	Mean (SD) BCVA visit 2 Untreated (letters n=10)	Mean (SD) BCVA visit 3 Treated (letters, n=9)	Mean (SD) BCVA visit 3 Untreated (letters n=9)	Mean (SD) BCVA visit 4 Treated (letters n=10)	Mean (SD) BCVA visit 4 Untreated (letters n=10)
59.8 (16.1)	77.1 (8.3)	63.9 (14.6)	79.1 (7.3)	68.3 (12.0)	78.0 (5.3)	68.6 (14.8)	79.3 (4.4)

Table 25. Treated and untreated mean and standard deviation (SD) visual acuity (ETDRS letters) for the participants 1-10 over all 4 sessions, visit 1 (baseline) to visit 4. The number of sessions analysed (n) was 10 in all cases except for visit three when the figure was 9 due to missing data.
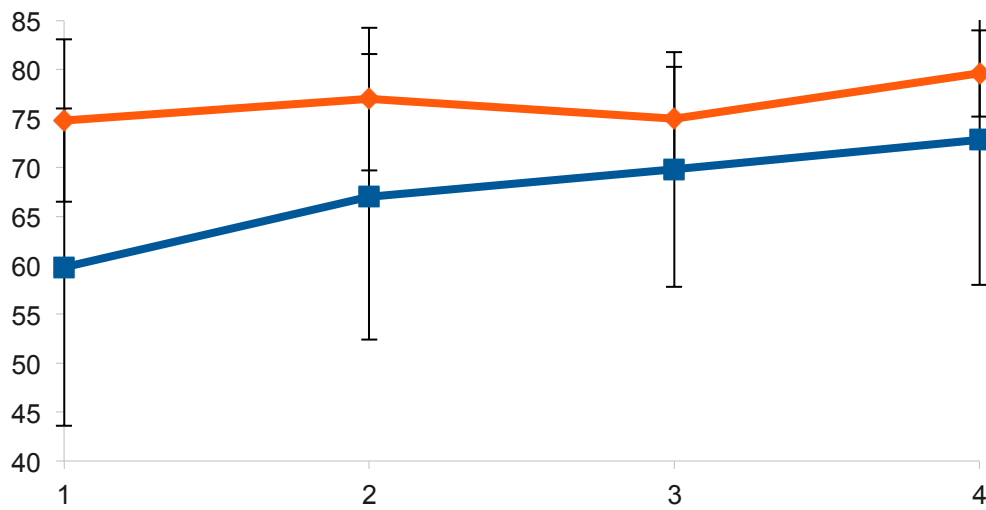
Figure 14. Mean (standard deviation) visual acuity (y-axis, ETDRS letter score) at visit 1 (baseline) to visit 4 (x-axis) for the 10 participants. Treated data is displayed in blue and untreated data in red. See Table 23.

6.5.2 Central Retinal Thickness

The CRT values for individual participants and the means of the cohort are presented below.

Participant number	CRT visit 2 Treated (μm)	CRT visit 2 Untreated (μm)	CRT visit 3 Treated (μm)	CRT visit 3 Untreated (μm)	CRT visit 4 Treated (μm)	CRT visit 4 Untreated (μm)
1	357	261	305	249	294	264
2	186	235	203	234	221	233
3	289	291	278	291	274	291
4	278	246	299	245	252	144
5	294	285	270	281	278	280
6	260	265	259	261	263	160
7	306	316	333	318	285	317
8	234	238	233	235	231	236
9	219	264	217	265	209	268
10	375	318	345	313	306	314

Table 26.   Treated and untreated CRT (central $1mm^2$ subfield retinal thickness, micrometres) for the participants, 1-10 over the 3 follow up sessions.

Mean (SD) CRT Baseline Treated (μm)	Mean (SD) CRT Baseline Untreated (μm)	Mean (SD) CRT Visit 2 Treated (μm)	Mean (SD) CRT Visit 2 Untreated (μm)	Mean (SD) CRT Visit 3 Treated (μm)	Mean (SD) CRT Visit 3 Untreated (μm)	Mean (SD) CRT Visit 4 Treated (μm)	Mean (SD) CRT Visit 4 Untreated (μm)
338 (145.5)	271 (29.9)	279 (56.7)	271 (29.5)	274 (47.5)	269 (30.6)	261 (32.4)	270 (29.9)

Table 27.   Treated and untreated mean and standard deviation (SD) CRT (central $1mm^2$ subfield retinal thickness, micrometres) for the participants 1-10 over all 4 sessions, visit 1 (baseline) to visit 4.
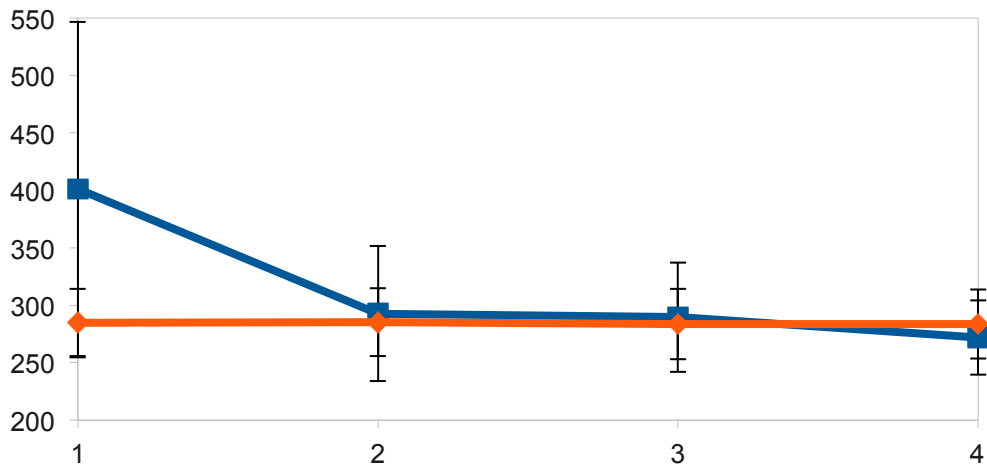
Figure 15. Mean (standard deviation) 1mm^2 central subfield retinal thickness (y-axis, micrometres) at visit 1 (baseline) to visit 4 (x-axis) for the 10 participants. Treated data is displayed in blue and untreated data in red.

6.5.3 Vector mean projected amplitude of BOLD responses

The BOLD response for individual participants and the means of the cohort are presented below.

Participant number	BOLD visit 2 Treated (n=8)	BOLD visit 2 Untreated (n=8)	BOLD visit 3 Treated (n=9)	BOLD visit 3 Untreated (n=9)	BOLD visit 4 Treated (n=8)	BOLD visit 4 Untreated (n=8)
1			0.474	0.746	-0.149	0.915
2	1.024	1.659			1.622	1.657
3	0.361	0.247	0.428	0.522	0.578	0.497
4	0.285	0.412	0.019	0.412		
5			-0.147	0.143	0.106	0.161
6	1.862	1.554	1.312	1.713	1.761	1.609
7	0.618	0.868	0.669	0.918	0.813	0.614
8	0/152	0.333	0.763	0.680	0.472	0.586
9	0.454	0.622	0.388	0.589		
10	0.112	-0.628	-0.263	-1.790	-2.622	-1.621

Table 28. Treated and untreated BOLD (vector mean projected amplitude of BOLD responses) for the participants, 1-10 over the three follow up sessions. A blank cell represents missing data. The number of participants analysed (n) was less than ten across all sessions after baseline due to missing data.

Mean (SD) BOLD Baseline Treated (n=10)	Mean (SD) BOLD Baseline Untreated (n=10)	Mean (SD) BOLD visit 2 Treated (n=8)	Mean (SD) BOLD visit 2 Untreated (n=8)	Mean (SD) BOLD visit 3 Treated (n=9)	Mean (SD) BOLD visit 3 Untreated (n=9)	Mean (SD) BOLD visit 4 Treated (n=8)	Mean (SD) BOLD visit 4 Untreated (n=8)
0.646 (0.644)	0.676 (0.604)	0.608 (0.584)	0.626 (0.753)	0.141 (1.123)	0.437 (0.888)	0.322 (1.459)	0.551 (1.025)

Table 29. Treated and untreated mean and standard deviation (SD) BOLD (vector mean projected amplitude of BOLD responses) for the participants 1-10 over the 3 follow up sessions. Following the baseline assessment, the number of participants analysed (n) was less than ten across the remaining sessions due to missing data.
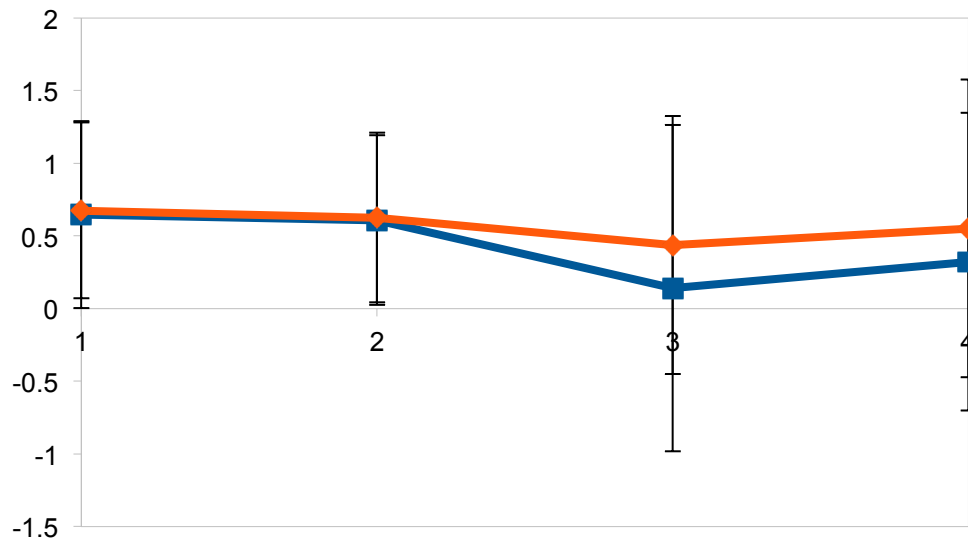
Figure 16. Mean (standard deviation) BOLD response (y-axis) at visit 1 (baseline) to visit 4 (x-axis). Treated data is displayed in blue and untreated data in red.

Participant number	BOLD (Treated / Untreated) Baseline (n=10)	BOLD (Treated / Untreated) visit 1 (n=8)	BOLD (Treated / Untreated) visit 2 (n=9)	BOLD (Treated / Untreated) visit 3 (n=8)
1	0.478		0.633	-0.163
2	1.006	0.617		0.979
3	0.938	1.458	0.820	1.186
4	0.279	0.692	0.046	
5	0.481		-1.030	0.657
6	1.683	1.200	0.766	1.940
7	0.693	0.712	0.729	1.323
8	0.289	0.458	1.218	0.805
9	0.781	0.730	0.659	
10	-0.699	-0.164	1.474	1.612

Table 30. Mean treated divided by mean untreated BOLD (vector mean projected amplitude of BOLD responses) for the participants 1-10 over the 3 follow up sessions. A blank cell represents missing data. Following the baseline assessment, the number of sessions analysed (n) was less than ten across the remaining sessions due to missing data.

Mean (SD) BOLD (Treated / Untreated) Baseline (n=10)	Mean (SD) BOLD (Treated / Untreated) visit 2 (n=8)	Mean (SD) BOLD (Treated / Untreated) visit 3 (n=9)	Mean (SD) BOLD (Treated / Untreated) visit 4 (n=8)
0.593 (0.615)	0.682 (0.484)	0.579 (0.715)	0.937(0.535)

Table 31. Mean and standard deviation (SD) treated / untreated BOLD (vector mean projected amplitude of BOLD responses) for the participants 1-10 over the four sessions, visit 1 (baseline) to visit 4. Following the baseline assessment, the number of sessions analysed (n) was less than ten across the remaining sessions due to missing data.
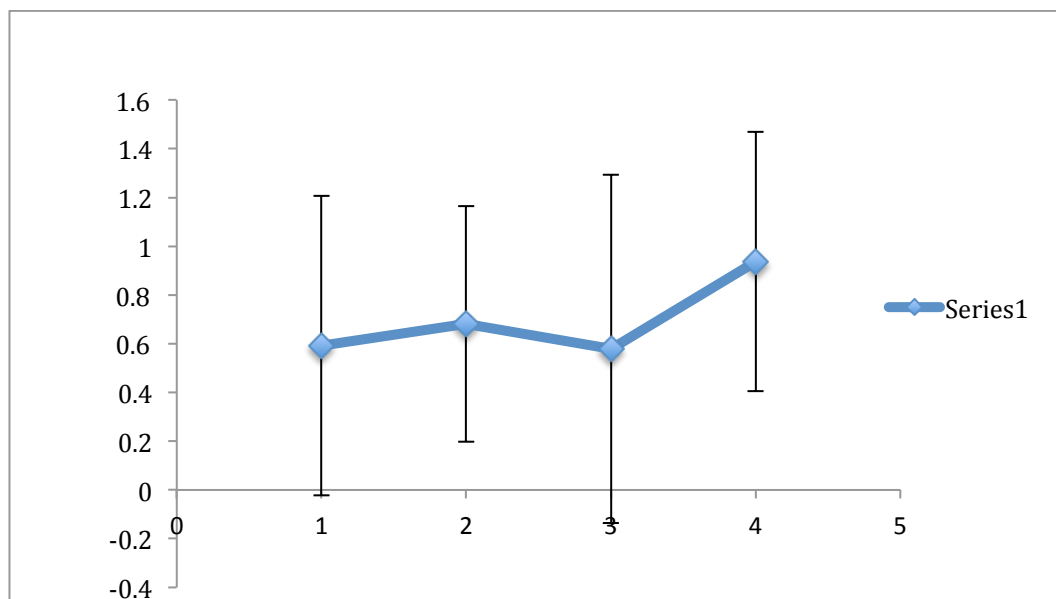
Figure 17. Mean BOLD response (treated over untreated, y-axis) at visit 1 (baseline) to visit 4 (x-axis).

6.5.4 Correlations between psychophysical and anatomical measures

The correlation between the treated eye VA and CRT was -0.473 (p=0.002). This was re analysed with the outlying results removed (VA 28 letters and CRT 738) leading to a correlation of -0.232 (p=0.155). The correlation between the BOLD (treated / untreated) and VA was 0.236 (p=0.142). Correaltion of the full set between BOLD and CRT was -0.410 (p=0.009). Removing the outlier (CRT 738 and BOLD -0.699) the correlation was -0.143 (p=0.385).
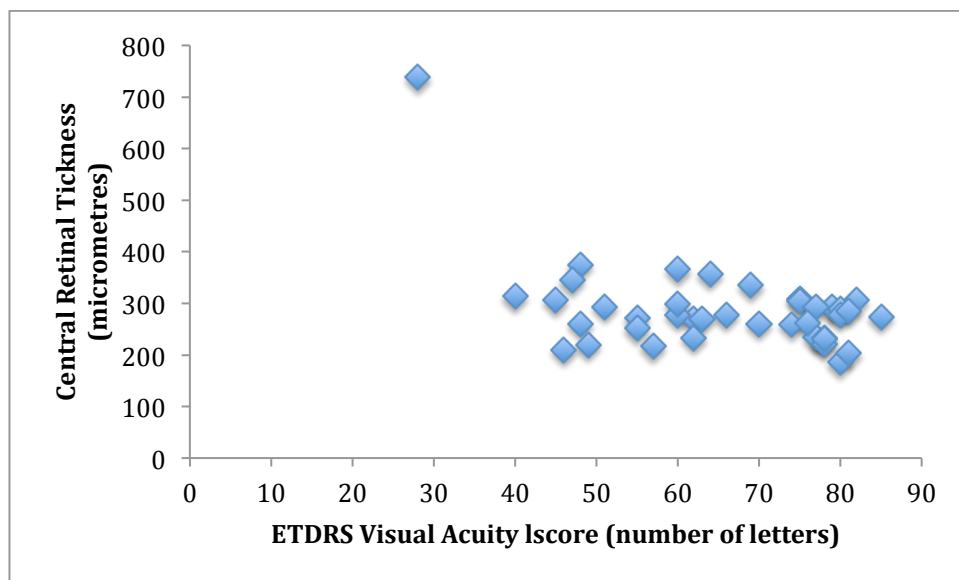
Figure 18. A scatter plot showing the relationship between the treated eye ETDRS Visual Acuity (number of letters) and the CRT (micrometres).
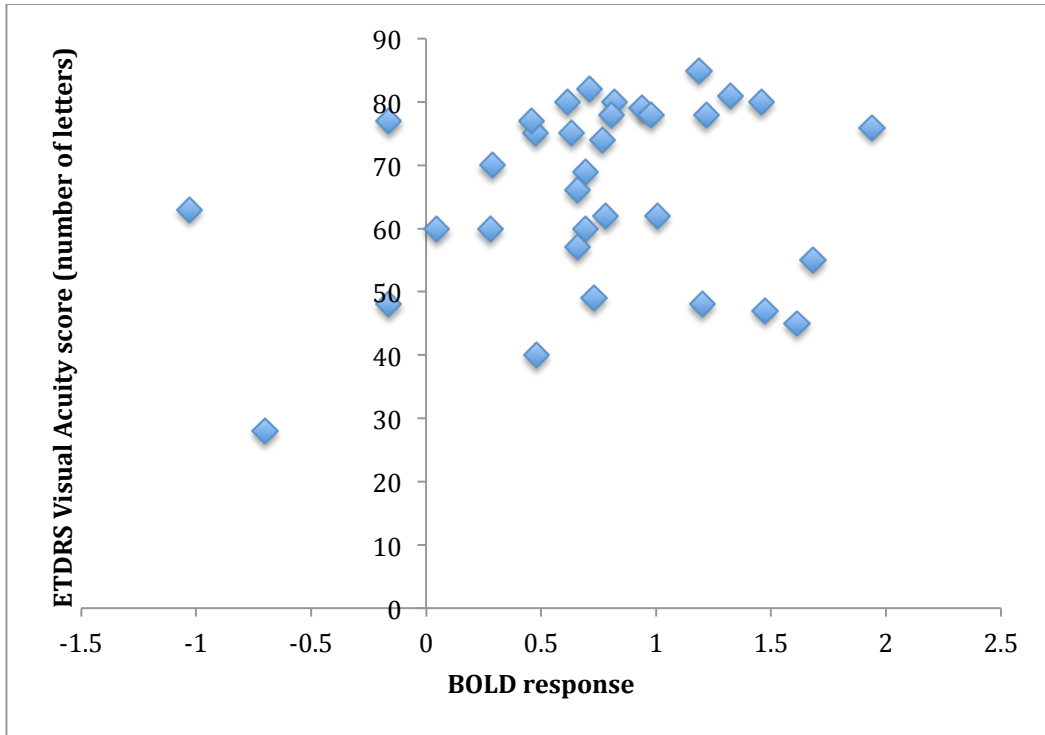
Figure 19. A scatter plot showing the relationship between the treated eye the BOLD response and the ETDRS Visual Acuity (number of letters).
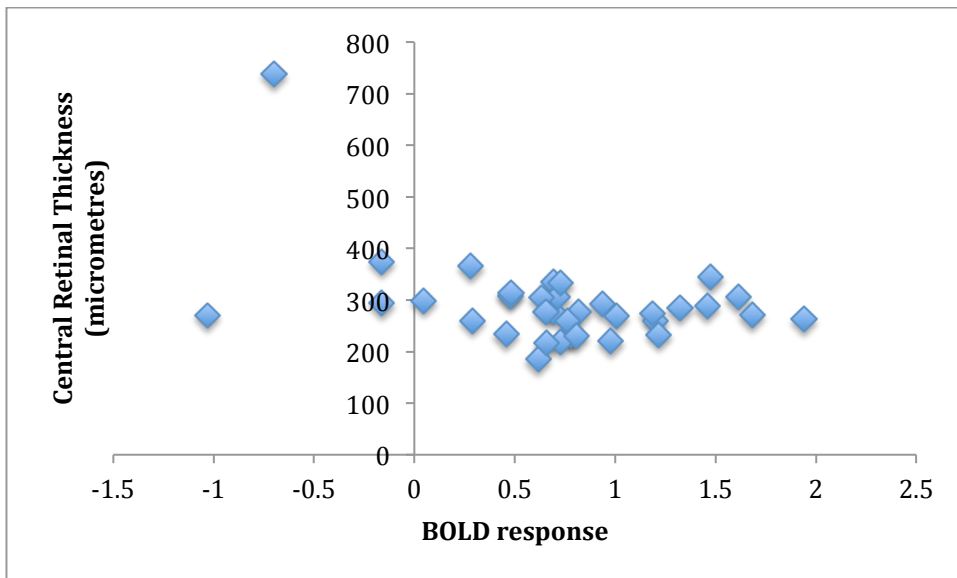
Figure 20. A scatter plot showing the relationship between the treated eye BOLD response and the CRT (micrometres).

6.6 Discussion

The mean VA improvement from pre treatment to after the third treatment of 8.8 ETDRS letters is in line with the expected response from larger published trials (Brown et al, 2011; Chakravarthy et al, 2013; Schmidt-Erfurth et al 2014). Similarly the corresponding reduction in central 1mm subfield CRT of 129.4 microns was smaller but similar to that expected. This demonstrates that our subject population is broadly representative of a typical population of nvAMD patients.

The BOLD response of both the treated and untreated eyes showed a mean decrease over the 4 sessions. At first glance this is surprisingly counterintuitive, but is a well-documented phenomenon, thought to be due to habituation or attenuation possibly because the same stimulus is presented at each session (Rosengarth et al, 2013). When the response of the treated eye was corrected for this expected variability by using the quotient of the untreated eye response, and the quotient of the MC, an improvement in activity was demonstrated from the first to the fourth session. This method of using a quotient is a logical step to take to counteract the habituation / attenuation effect. Just as Rosengarth had used during many of her experiments, the method used in this thesis employed a full field as a stimulus at each visit. This aimed to give consistency across sessions, but ultimately may have worked against the ability to show a greater effect. To counteract the habituation / attenuation effect an alternative method would have been to use either a stimulus appearing in multifocal locations over a short time sequence or to use randomly selected different patterns during and between sessions. Consideration needs to be given to this for future studies. In a similar vein the VA and CRT data could have been presented using quotients of treated eye divided by untreated eye, but because there are relatively small standard deviations associated with the mean values of the treated eyes this calculation seems unnecessary.

Nonetheless, using the quotient the rise in BOLD is consistent with the effect shown in Baseler et al's single case report (Baseler et al, 2011). It supports the notion that the area of the primary visual cortex anatomically mapped to and so representing the central macula is able to increase its activity after a period of having reduced activity due to nvAMD. A degree of caution has to be applied to this conclusion though as the standard deviations are very large. It may be that a non-parametric statistic would capture the overall trend in BOLD response change without displaying such high variance in the absolute quotient and this type of analysis should be considered as a pre-planned outcome measure for future studies. The increase in cortical activity following treatment is an important finding in its own right but also allows the discussion about the place of using the BOLD response as an outcome measure of the treatment of AMD. The significant variability in the single subject BOLD response between sessions indicates that it is not a good measure of individual response to treatment from visit to visit. When a cohort is studied the mean change in BOLD is less vulnerable to this variability and so it is better to use mean values to demonstrate treatment effect. This conclusion is similar to that of the use of VA in chapter 1, in that as an outcome measure it is also open to significant intersession coefficient of variability (Patel et al, 2008; Aslam et al 2014).

What cannot be demonstrated is if the recovery of visual cortex function is back to its fully pre diseased state as we do not have BOLD data prior to the onset of the neovascularisation. One would estimate that there would be full recovery if the retinal function allowed it, and can extrapolate thinking from other disease processes. From studies of patients treated for nvAMD it is known that VA does not return to the pre-diseased level (Airody et al, 2014; Tufail et al, 2014), but this is likely to be due to the incomplete amelioration of the retinal pathology as we see full visual recovery following treatment of other causes of reversible visual loss such as following cataract surgery. It has already been discussed that anatomical changes can occur in the grey matter in those having developed AMD and those with longer term retinal lesions (Noppeney et al, 2005; Boucard et al, 2009; Hernowo et al 2013).

Following on from this point, it is also not possible to conclude if there is a critical window of opportunity to protect the visual cortex from an inability to recover activity either fully or partially. This knowledge would have implications on the timing of treatments to try to recover vision following the onset of nvAMD and furthermore whether neuro-protection strategies in new onset disease would be advisable.

This study was open to bias in a number of areas in particular in the subjective selection of the Regions of Interest (ROIs). The ROIs were determined by selecting what was estimated to be the correct anatomical locations corresponding to the macula and to a region in the peripheral retina without any knowledge of their function. It is possible that the posterior pole and to a lesser extent the mid calcarine regions selected were not of the correct location, size or shape and therefore do not show the full effect of the change. To counteract this the ROIs could be selected to find the regions that have the greatest increase when the raw BOLD data is visualised, but this technique could be criticised in that one could 'select the result to fit the question'. An alternative is to use line ROIs that join the PP and MC areas as done by Smirnakis et al. This captures a much larger cross section of the cortex which means that the location of the change in the BOLD response and not just its magnitude become the important end points (Smirnakis et al, 2005). Further consideration should also be given to using larger regions of interest, even possibly the whole visual cortex, which may increase the chance of capturing change in activation.

Another area of bias was that by the very nature of the duration and commitment required for fMRI, and particularly so in this older population, not all participants were able to attend all sessions. This could compromise the quality of the data. However given that only 5 sessions were missed in a total of 40, and that no participant missed more than one session, the results are likely to be representative of a full dataset. The fact that participants were not always able to attend their session as well as the cost, duration and contraindications for the use of MRI, strengthens the argument that fMRI is

not a suitable tool for an assessment tool to guide re-treatments. The reasons listed would have also contributed to slow recruitment into the study. Once again, all of these factors need to be taken into consideration for future studies.

Correlations between the VA, CRT and BOLD response all missed significance, which could be due to the under powering of the study, but may also be due to the techniques being insufficiently sensitive to detect small change.

Further work need to be done to refine the technique of both detection and analysis of the BOLD response, in order to increase its sensitivity. Although using the mid calcarine response and the fellow eye to control for the variation in the BOLD response seems logical, this quotient is in fact a ratio of a ratio and therefore has significant disadvantages. It may be an inherently unstable value particularly when there are large changes in the denominators. In addition to the drawback it may not be valid to use parametric analyses. Furthermore the use of negative values (as driven by some negative BOLD responses) appears intuitively inappropriate. These are all useful learning points for the design of future outcome analyses using the BOLD response.

6.7 Conclusion

The results of this study quite substantially confirm that the area of the primary visual cortex anatomically mapped to and so representing the central macula is able to increase its activity after a period of having reduced activity due to nvAMD. Furthermore measuring the BOLD response by fMRI appears to have potential to be a good, objective way assessing cortical function in response to treatment. However, due to its intersession variability and for practical reasons, the technique described in this thesis is best placed to assess cohorts of individuals, retrospectively over multiple scans, and at the moment in the research setting.

Chapter 7. Quantifying the adverse effects of macular degeneration treatment: Short term intraocular pressure trends following intravitreal Ranibizumab injections for neovascular Age-related Macular Degeneration and the role of oral Acetazolamide in protecting glaucoma patients

7.1. Introduction

The preceding chapters have focused attention on measuring the efficacy outcomes of the treatment of neovascular macular degeneration (nvMD). An equally important consideration of any treatment is the quantification of adverse events. This chapter studies the risk and implications of short term raised intraocular pressure (IOP) as a side effect of treatment of neovascular age-related macular degeneration (nvAMD) with intravitreal ranibizumab. Furthermore the prevention of raised IOP with the use of oral acetazolamide is evaluated in a randomised controlled trial.

7.2. Background

As has been highlighted in previous chapters, nvAMD contributes substantially to the visual loss burden in society (Owen et al, 2012). Following a ruling by the National Institute of Health and Clinical excellence (NICE) in 2008, routine treatment is widely available in the UK and on average enables partial restoration of vision and its continued preservation in the longer term (NICE TA155, 2008; Rofagha et al, 2013; Tufail et al, 2014; Airody et al, 2014; Schmidt-Erfurth et al, 2014).

All therapeutic interventions have associated side effects or complications and intravitreal injections with anti-VEGF agents are no exception to this rule. The most feared complications are as a result of structural damage to the eye at the time of injection or as a result of an infection affecting the internal coats of the eye manifesting in the post injection period, a process termed endophthalmitis. Endophthalmitis often leads to a devastating

outcome on final visual acuity and routine precautions such as prophylactic antiseptic treatments and a strict aseptic environment are routinely employed (Boyer, Heier, Brown, Francom & Ianchulev, 2009; Hasler et al, 2014). These events, although uncommon, have been well studied and occur at a frequency of less than 1 in a 1000 injections, with endophthalmitis at approximately 1 in 3000 injections, (Brown et al, 2006; Rosenfeld et al, 2006; Boyer et al, 2009; Martin et al, 2011; Chakravarthy et al 2012).

A less well-studied side effect is the phenomenon of short term raised IOP following injection into the vitreous cavity. This unwanted effect is particularly relevant to patients who have glaucoma, a condition whereby there is progressive loss of the retinal nerve fibre layer manifesting as a 'cupped' appearance of the optic nerve. This loss causes peripheral visual defects and as the condition progresses towards an advanced stage field loss encroaches on and then finally obliterates the nerve responsible for fixation. The principle risk factor for glaucoma is raised IOP, which untreated is usually above the normal range of 10-22mmHg. Treatment is aimed at pharmacologically, and occasionally surgically, lowering the IOP and reducing the diurnal variation of the pressure.

At the time of an intravitreal injection of a therapeutic dose of and anti-VEGF agent (usually 0.05mls) a sharp rise in IOP occurs, raising it to a mean value of around 44mmHg. After 30 minutes the IOP usually subsides to around 30mmHg (Kim Manravadi, Hur & Covert, 2008; Falkenstein, Cheng & Freeman, 2007; Hollands, Wong, Bruen, Compbell, Sharma & Gale, 2007). As commented upon by Mathalone et al, this effect may not be transient. In approximately 11% of 201 eyes treated with bevacizumab the IOP rise was sustained, being defined at IOP greater than 22mmHg or a rise of 6mmHg or more from baseline for at least 30 days. Retreatment intervals of less than 8 weeks were found to be a risk factor (Mathalone et al, 2012).

With the knowledge that nvAMD is a chronic disease requiring multiple injections over a number of years (Rofagha et al, 2013; Tufail et al, 2014;

Airody et al 2014), concern has been raised as to the whether peripheral visual loss may be being promoted in those who have glaucoma and are being treated for nvAMD. Loss of peripheral vision has particular implications for those being treated for nvAMD who, by NICE guided criteria, must already have compromised central vision (Kim et al, 2008; Falkenstein et al, 2007). Furthermore debate exists as to whether those with glaucoma are more vulnerable to IOP rise following injection. In Kim et al's study of 213 consecutive injections in 120 eyes a mean IOP immediately post injection of 44 mmHg (range 4 to 87 mmHg) was observed. Twenty of these patients had pre-existing glaucoma and it was noted that these glaucomatous eyes took significantly longer to return to a pressure of 30 mmHg or lower (Kim et al., 2008). To support the opposing view Frenkel et al studied 22 patients receiving intravitreal pegaptanib injections (a less efficacious and so now a less commonly used anti-VEGF agent) and showed no statistical significance between the 2 groups at any time interval. This study however only had 9 glaucoma patients and there was no clarity about which of the patients may have received prophylactic IOP lowering treatment (Frenkel et al., 2007).

Similarly it is not clear if treatments to prevent raised IOP are useful. Frenkel et al reported upon another series of 71 patients being injected with one of three different types of anti-VEGF agents: ranibizumab, bevacizumab and pegaptanib. Various types of prophylactic IOP lowering medication (the anti-glaucoma drops timolol, brimonidine, aproclonidine and brinzolamide or oral acetazolamide) were administered 1-2 hours prior to injection in around two thirds of cases.   The report concluded that glaucoma patients behaved in a similar manner to non-glaucoma patients and that IOP-lowering medications were essentially ineffective (Frenkel, Haji & Frenkel, 2010). The non-randomised and inconsistent use of medications in this study casts doubt upon the validity of such a bold statement. Theoulakis et al however, reported on a prospective double-blind placebo controlled study and concluded that use of Combigan (brimonidine and timolol) twice a day on the day before an intravitreal injection of Ranibizumab is effective.

Unfortunately on this occasion IOP was not measured immediately post injection missing the highest IOP spike (Theoulakis et al., 2010). All of these studies mentioned so far did not specifically study the population most at risk to losing peripheral visual field: those with glaucoma or glaucoma suspect.

Any loss of the nerve fibre layer and associated visual field with single IOP spike is likely to be too small to be detectable with current technology and so ideally very long term studies of these indices should be performed. As with the studies described so far in this chapter raised IOP is often taken as a surrogate marker of potential visual field loss and so is the focus of investigation (McMonnies, 2008; Kim et al, 2008; Falkenstein et al, 2007; Frenkel et al, 2007; Frenkel et al, 2010). The aim of the study in this chapter is to determine the effect of a single prophylactic medication, oral acetazolamide, on lowering the peak and duration of IOP rise in glaucoma and glaucoma suspect patients, following an injection of ranibizumab for nvAMD. Oral acetazolamide was chosen because of it known properties in being able to clear volume from so called 'fourth spaces' such as the vitreous and its already established use in some nvAMD treatment clinics.

7.3. Methods

In accordance with the Declarations of Helsinki, York Teaching Hospital NHS Foundation Trust gave approval for the study and the trial was assigned EudraCT (European clinical trial database) number 2010-023037-35.

7.3.1 Participant selection

Participants were recruited from a single centre nvAMD treatment clinic, had to have been previously diagnosed with glaucoma or glaucoma suspect and to require an intravitreal injection of ranibizumab in accordance with NICE guidance (NICE TA155, 2008). The full inclusion and exclusion criteria are listed in Table 32.

Inclusion criteria:
1. Patients with nAMD requiring Ranibizumab injections.
2. Glaucoma or glaucoma suspect.
3. Able to give written informed consent.
Exclusion criteria:
1. Baseline pre-injection IOP of 30 mmHg or higher.
2. Known allergy to sulphur/sulphonamide containing drugs or acetazolamide.
3. Severe kidney or liver disease/dysfunction.
4. Adrenal gland failure.
5. Hyperchloraemic acidosis.
6. Hepatic cirrhosis.
7. Pregnancy/Pre-menopausal.
8. Concomitant use of other oral carbonic anhydrase inhibitors.
9. Enrolment in a pre existing clinical trial

Table 32. The inclusion and exclusion criteria for participation in the IOP rise prophylaxis study.

7.3.2 Trial design

Participants were randomised 1:1 to receive either 500mg acetazolamide or no treatment 60-90 minutes prior to treatment. The randomisation sequence had previously been software generated by an independent member of staff and was held by the trial pharmacist. In accordance with the Royal College of Ophthalmologists guidance on administration of intravitreal therapies, 0.5mg in 0.05mls of ranibizumab was administered via the pars plana (RCOphth, 2009). The IOP was measured using a Tono-Pen (Medtronic Xomed Ophthalmics Inc., Mineapolis, Minnesota, USA) prior to injection to give the baseline (TB) reading. A second member of the

research team verified the reading on the Tono-Pen screen. Further readings were taken immediately after the injection (T0) and subsequently at 5 (T5), 10 (T10) and 30 (T30) minutes post treatment.

7.3.3 Statistical methodology

The primary end points were change in IOP from baseline to time points T0, T5, T10 and T30. No published data existed on the anticipated treatment effect. An effect of 9mmHg difference at T0 was therefore estimated based upon clinical experience of the use of acetazolamide in other conditions with raised IOP by three ophthalmologists. To provide a power of 80% with a 5% significance level 12 participants in each arm were required.

An intention to treat analysis was used, with repeated measures ANOVA to compare IOP over time and ANCOVA to compare between the arms at each time point. All analyses were adjusted for baseline IOP. All analyses were undertaken on SPSS version 18.0 (IBM, Portsmouth, UK) and $p < 0.05$ was considered to indicate statistical significance.

An exploratory endpoint was to observe any treatment differences in the subtypes of glaucoma at baseline. These baseline observations along with age, sex and race were summarised using means (standard deviations), medians (inter-quartiles ranges) and proportions (percentages).

7.4 Results

Twenty-four participants were randomised and completed the study. Their baseline characteristics are displayed in Table 33.

		No aceta-zolamide (12)	Aceta-zolamide (12)	Total
Age	Mean (SD)	84.9 (7.4)	80.8 (4.6)	82.9 (6.4)
Gender	Male	5 (42%)	9 (75%)	14 (58%)
	Female	7 (58%)	3 (25%)	10 (42%)
Ethnic group	Caucasian	12 (100%)	12 (100%)	24 (100%)
Eye injected with Lucentis during study	Right eye	7 (58%)	6 (50%)	13 (54%)
	Left eye	5 (42%)	6 (50%)	11 (46%)
Type of Glaucoma or suspected glaucoma	Normal tension glaucoma	0 (0%)	2 (17%)	2 (8%)
	Primary / Chronic open angle glaucoma	10 (83%)	8 (67%)	18 (75%)
	Angle closure glaucoma	1 (8%)	0 (0%)	1 (4%)
	Glaucoma Suspect	1 (8%)	2 (17%)	3 (13%)
Trabeculectomy	No	11 (92%)	10 (83%)	21 (88%)
	Yes	1 (8%)	2 (17%)	3 (12%)
Cataract surgery	No	6 (50%)	7 (58%)	13 (54%)
	Yes	6 (50%)	5 (42%)	11 (46%)
Number of concomitant medications for glaucoma	0	1 (8%)	2 (17%)	3 (13%)
	1	7 (58%)	5 (42%)	12 (50%)
	2	4 (33%)	5 (42%)	9 (38%)

Table 33. Baseline characteristics of study participants in the acetazolamide treated and non-treated groups.

A reduction in IOP was demonstrated from T0 to T30 ($F_{(4)}=72.97$, $p<0.001$), although no difference between the two groups was observed ($F_{(1)}=0.57$, $p=0.459$). Data is displayed in Table 34 and graphical representation in Figure 18.

		Mean (SD)	Range	95% CI
Baseline (TB)	No Acetazolamide	15.1 (5.5)	[8-26]	12.0, 18.2
	Acetazolamide	15.8 (4.8)	[8-26]	13.1, 18.4
Post Lucentis injection at 0 minutes (mmHg)	No Acetazolamide	44.5 (19.8)	[19-86]	33.3, 55.7
	Acetazolamide	42.2 (10.2)	[25-58]	36.4, 48.0
Post Lucentis injection at 5 minutes (mmHg)	No Acetazolamide	31.4 (14.4)	[13-65]	23.3, 39.6
	Acetazolamide	27.1 (10.0)	[14-48]	21.4, 32.7
Post Lucentis injection at 10 minutes (mmHg)	No Acetazolamide	24.5 (11.7)	[10-50]	17.9, 31.1
	Acetazolamide	21.3 (7.0)	[10-35]	17.4, 25.3
Post Lucentis injection at 30 minutes (mmHg)	No Acetazolamide	20.6 (9.5)	[11-46]	15.2, 25.9
	Acetazolamide	15.7 (4.3)	[8-21]	13.3, 18.2

Table 34. Intraocular pressures in the acetazolamide and control group over each time point summarised with descriptive statistics.
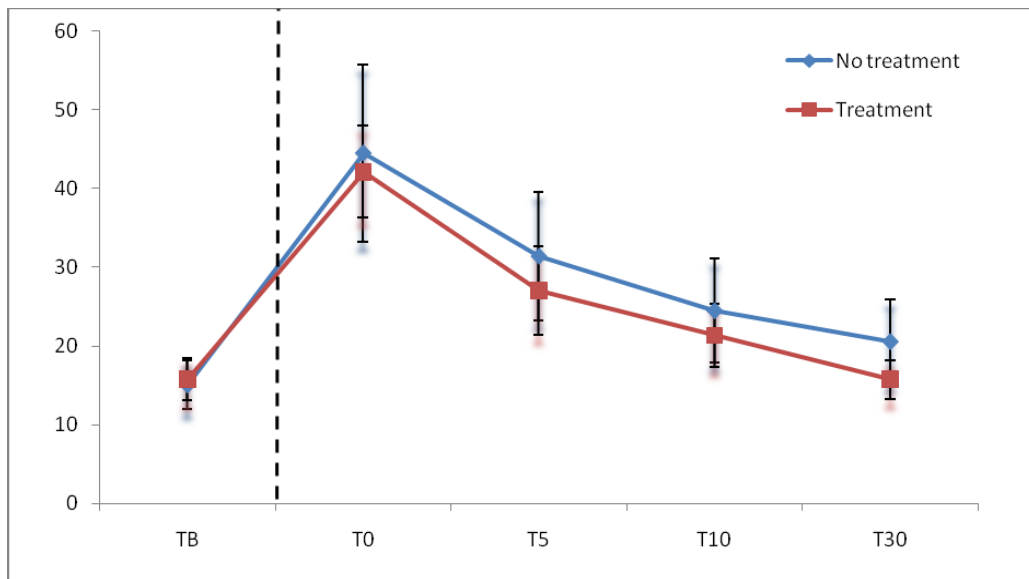
Figure 21. Mean (SD) intraocular pressure (mmHg, y axis) for time points TB, T0, T5, T10, T30 (minutes, x axis). The dashed line indicates the injection occured between TB and T0

Single time points were analysed in an attempt to reveal data for potential future studies, such as observing IOP over a longer duration. No difference was seen between the two groups at time points T0, T5 or T10 but a difference at T30 was noted (SD 0.1, 95% CI 1.3-9.8, p= 0.013). Data for these time points is displayed in Table 35.

	Absolute change from TB		Difference between groups adjusting for baseline Mean (SD) (95% CI)	ANCOVA p-value
	No Acetazolamide (SD)	Acetazolamide (SD)		
Change from TB to T0	29.4 (16.1)	26.4 (8.2)	3.8 (0.4) (-6.2, 13.7)	0.440
Change from TB to T5	16.3 (10.0)	11.3 (9.7)	5.4 (0.2) (-2.7, 13.5)	0.183
Change from TB to T10	9.4 (8.5)	5.6 (6.0)	4.0 (0.2) (-2.3, 10.3)	0.201
Change from TB to T30	5.5 (5.4)	0.00 (4.4)	5.5 (0.1) (1.3, 9.8)	0.013

Table 35. A comparison in the change in IOP from baseline to T0, T5, T10 and T30 between the treated and untreated groups.

A post-hoc analysis of area under the curve was calculated for the two groups: 780.4 for the group not receiving acetazolamide and 665.0 for the treated group.

Throughout the short time participants were enrolled in this study no serious adverse or adverse events were reported.

7.5 Discussion

This study shows that transient IOP increase following intravitreal injection of ranibizumab for nvAMD is commonplace and can be substantial in glaucoma and glaucoma suspect patients. These results are in line with previous studies that have not specifically followed glaucoma patients (Frenkel et al, 2007; Kim et al, 2008; Schmidt-Urfurth, 2010; Frenkel et al,

2010) although debate continues as to whether IOP behaves differently following an injection in those with glaucoma compared to those without (Kim et al, 2008, Frenkel et al, 2010). Nonetheless, given that IOP spikes are a risk factor for the development and progression of glaucoma (McMonnies, 2008), and understanding that a substantial increase in IOP occurs with injections (and potentially worse still as treatments may be up on a monthly basis), precautionary measures must be considered in this at risk population.

The study in this chapter used oral acetazolamide as prophylactic treatment to try and address the issue of prevention of IOP rise following intravitreal injection. The treatment and non-treatment groups were well balanced for baseline characteristics, although the non-treatment group was slightly older and had a female propensity, so any differences in effect are likely to be due to the treatment. Overall, no statistical significant difference was observed between the group that received acetazolamide and the group that did not. In an attempt to look for data that may be useful to be able to power future studies an analysis of the single time points were performed. A statistically significant reduction in post injection IOP spike was seen at T0, T5 or T10 but a difference was observed at T30 (20.6mmHg versus 15.8mmHg). At first assessment the results therefore imply that the peak IOP is not affected by the treatment but the duration of IOP rise is shortened. This finding would need further examination to be confirmed. It can be argued that a modest reduction in IOP at 30 minutes may not be clinically significant in all but those patients with the most vulnerable retinal nerve fibre layers.

There are two mechanisms whereby raised IOP can potentially cause nerve fibre layer damage; peak IOP and sustained raised IOP. Without the benefit of having measured the nerve fibre layer thickness at these time points using optical coherence tomography (and assuming the degree of change would in fact be measurable after a single injection), it is not easy to discern which of these factors is the most important. It may well be that both play a

role and that the IOP reduction in the area under the curve is important (Figure 18). Post hoc analysis showed a numerical difference suggesting that this could be a candidate measure for future studies. Furthermore an interesting observation to note is that at each time point the range of measured IOPs and the peak pressure was substantially lower in the treated group; this reduced chance of a very high peak pressure may be of more clinical significance in protecting the nerve fibre layer than the mean reduction in IOP and so the effect of the prophylactic acetazolamide may be more than it first appears. A larger sample size may have been able to detect this difference.

If clinicians choose to use acetazolamide as prophylaxis against IOP rise they will need to debate the clinical significance of these findings. In particular there is a need to balance the apparent limited degree of efficacy with the potential side effects of treatment. Although no adverse events were reported in this study, acetazolamide can precipitate renal impairment and confusion, particularly in the elderly, which is of course the nvAMD population.

Analysing the exploratory outcome of differences between the groups based upon the type of glaucoma was not valid as by far the majority of participants had primary open angle glaucoma.

The design of the study in this chapter was robust in all but the method of informing the power calculation. Perhaps a larger group of clinical experts may have decided upon a lower peak reduction in IOP and so a larger sample size would be been used: the study appears under-powered. Nonetheless the results of this study act as good pilot data to inform future studies. Furthermore, proving a statistical significance may not necessarily change its clinical relevance.

Prophylactic medical treatments are unlikely to be able to prevent the full extent of the IOP spike as the globe is principally a rigid structure. A positive

correlation has been described between rigidity coefficient and age suggesting that injection into an older more rigid eye would produce a higher peak IOP (Pallikaris, Kymionis, Gnis, Kounis & Tslimbaris 2005; Kotliar et al, 2007). Other factors have also been found to contribute to the degree of IOP rise such as the volume of the eye, needle bore size, vitreous reflux, and volume injected (Kim et al, 2008; Kotliar et al, 2007). In some eyes that are the most vulnerable to an IOP spike the only way to prevent an IOP rise may be to remove the equivalent volume from the eye immediately prior to injection. This is not a published technique and has the additional risk of requiring two punctures.

7.6 Conclusion

This chapter reports on the first randomised controlled trial studying the prophylaxis of IOP rise following intravitreal injection in glaucoma and glaucoma suspect patients. Oral acetazolamide given 60-90 minutes before ranibizumab injection to treat nvAMD reduced mean IOP 30 minutes post injection by approximately 5 mmHg. Although this figure in itself it likely to be of little clinical significance, the reduction in peak IOP values immediately following injection and the reduced 'area under the curve' may well be important to those most vulnerable to pressure rise.

8. Summary and Conclusions: Quantification of neovascular macular degeneration treatment

This thesis began by discussing macular degeneration and with particular emphasis on the neovascular form and its treatments. The common and some novel ways of quantifying treatment effect were then examined in a series of 6 studies. This final chapter summarises the context of the thesis, its findings and then discusses some alternative methods of assessing outcomes of treatments not studied here, before drawing final conclusions.

8.1 Overview of the context

Neovascular macular degeneration, and in particular the age-related form is a common cause of vision loss in the population (Owen et al, 2012). Treatments are now available in the form of regular anti-vascular endothelial growth factor (anti-VEGF) injections into the vitreous of the eye, which not only restore a degree of the visual loss but also maintain vision in the long term (Martin et al, 2011; Chakravarthy et al, 2012; Schmidt-Erfurth et al, 2014; Tufail et al, 2014; Airody et al, 2014). Measuring the outcome of treatments is an important aspect of any care, not only to document progression through the disease journey, but also to aid treatment decisions such as which therapies to use and when. Documenting outcomes are equally important when describing the response to or side effects of treatments in populations, as effects are not always necessarily predictable in individuals.

In a clinical setting the conventional way of reporting nvAMD treatment outcome is to use Visual Acuity (VA) (Brown et al, 2006; Rosenfeld et al, 2006; Tufail et al, 2014). There is much discussion about the merits of doing this, in particular that VA alone may not be a comprehensive representation of vision and indeed could underestimate visual function (Scott et al, 1994; West et al 1997; Mangione et al, 1999; Maclure et al, 2000; Hazel et al, 2000). Central retinal thickness (CRT) as measured by Optical Coherence

Tomography (OCT) is also used as a routine outcome measure usually to aid retreatment decisions (Lalwani et al, 2009; Martin et al, 2011; Chakravarthy et al, 2012; Airody et al, 2014). In a similar way to the VA discussion, there is also comment that the use of OCT alone may not fully represent visual function change with treatment (Alexander et al, 2012).

This thesis aimed to assess these common forms of outcome measures and in addition aimed to explore the use of some functional measures including the use of Patient Reported Outcome Measures (PROMs), microperimetry (MP) and functional Magnetic Resonance Imaging (fMRI). In line with this theme, not just the positive aspects of therapy were studied, but also a negative aspect in the form of the prevention of short-term pressure spikes following intravitreal injection.

8.2 Overview of the findings

The studies presented comprise observational, interventional, randomised and open label methodology.

8.2.1 Visual acuity as an outcome measure

At the time of this study the principle treatment for nvAMD was ranibizumab administered monthly for 3 months and then as required on a monthly basis. (Lalwani et al, 2009; NICE TA 155, 2008). As a part of service redesign, and in particular to try and ensure that patients received assessment on a monthly basis, a new treatment clinic was established. To evaluate the benefit of this change VA outcomes were assessed. Seventy-two eyes of 62 patients with nvAMD who had already received the 3 initiation treatments of ranibizumab and were in the so called 'stability phase' of their treatment were studied. Outcomes were assessed 6 visits before and 6 visits after their move to the new location (Tschour et al, 2013). In the 6 visits prior to transfer the patients were seen at a mean frequency of 56.8 days (8 weeks) and had a mean loss in VA of 1.1 ETDRS letters. Following

transfer the patients were seen at a mean frequency of 31.8 days (4.3 weeks) and subsequently VA improved by 4.6 letters. This information supports the notion that if their dosing regimen is to be on an 'as required' basis, patients with nvAMD should have better outcomes if they are seen monthly rather than 8 weekly.

In this case distance ETDRS letter score VA was used as the principle outcome measure. Its main advantages are that it is a quick and relatively simple assessment tool that produces a numerical value that can be used easily for statistical analysis. The principle disadvantages that were discussed were that it has a large co-efficient in repeatability (Patel 2008; Aslam, 2014) meaning that it is not good at representing true responses between visits, and that it is not a full reflection of visual function (Scott et al, 1994; West et al 1997; Mangione et al, 1999; Maclure et al 2000; Haze et al, 2000). Consequently basing re-treatment decisions on VA alone, as was suggested in the product license of one anti-VEGF treatment does not appear to be appropriate (SmPC Ranibizumab, 2007).

8.2.2 Central retinal thickness as an outcome measure

Pathological myopia (PM) is the second commonest cause of macular degeneration and affects a younger population than its age-related counterpart. Neovascular disease is a common complication of PM and, left untreated, has a poor natural history (Chan et al, 2008; Montero et al, 2010). At the time of study there was no licensed anti-VEGF treatment for the treatment of CNV secondary to PM. The REPAIR study was aimed at evaluating the safety and efficacy of ranibizumab in the treatment of CNV secondary to PM. The local dataset was combined with a national data set and showed that there was a mean improvement of 13.8 (SD 14.0) ETDRS letters at 12 months with a median number of treatments being 3 injections. The CRT as measured by spectral domain OCT, decreased by a mean of 108μm (SD 109) at this time point.

The principle advantages of using CRT are that the information is quickly acquired by OCT, it is highly reproducible (Giana, Deiro & Staurenghi, 2012) and a numerical value enables statistical manipulation. The CRT may not give complete information about the activity of neovascular disease however, as this may be governed by the exact site of the thickening and exactly what is measured within the 'central retinal' area. Furthermore, to aid re-treatment decisions qualitative aspects of the OCT scan are important (Martin et al, 2012; Chakravarthy et al, 2012) and training of those who interpret the image is required to given consistent results (Joeres et al, 2007).

8.2.3 The use of Patient Reported Outcome Measures

Quality of life measurements give a holistic approach to outcomes of treatment and have been suggested to be more important than clinical outcomes (Deshpande et al, 2011). Chapter 4 used the same REPAIR cohort of individuals as in the CRT study but analysed outcomes using a well-being questionnaire (W-BQ12) (Riazi et al, 2006) and a treatment satisfaction questionnaire (MacTSQ) (Mitchell et al, 2007). The results showed that individuals had a small but statistically significant improvement in their well-being and an improvement in treatment satisfaction over the 12 month study period.

Both the W-BQ12 and MacTSQ only correlated weakly with mean VA improvement in the cohort. This gives hope that a specifically powered study would give validity to these tools and also that VA could act as a biomarker for quality of life under this circumstance. PROMs give a new dimension to the assessment of outcomes and intuitively a change in quality of life measurement is more relevant to those receiving the treatment than a surrogate biomarker. Collecting and interpreting W-BQ12 and MacTSQ data is time consuming and not practical for all patients in a high volume clinical setting, however. PROMS are well placed to retrospectively evaluate interventions, but given their nature they do not directly help treatment

decisions. At present they are best placed as a research or service evaluation tool.

8.2.4 The use of microperimetry as tool for outcome measurement

Data from eight participants undergoing routine, NICE approved NHS care for treatment of nvAMD were analysed in the study. All eight had VA, OCT and microperimetry (MP) assessments followed by treatment with anti-VEGF therapy at baseline. Visual acuity, CRT and MP were repeated prior to re-treatment at two further subsequent monthly visits. Due to missing data only the first three time points were analysed. The mean VA improved by 11 ETDRS letters and the CRT decreased by 61 micrometers.

8.2.4.1 Retinal sensitivity

Retinal sensitivity (RS) was analysed from within an 'area of interest' that was defined as the area of CNV and sequelae guided by late frame FFA and OCT images. The percentage of points within the area of interest that increased by 2dB or more or 4dB or more was used as the principle outcome measure. One month following the first treatment a mean of 33% of points increased by 2dB or more and 18% by 4dB or more. At two months, following the second treatment the figures rose to 62% and 39% respectively.

Despite the main set back of this study being low participant numbers and a short study duration (12 months would have been better), RS has the potential to be used as an outcome measure not only for populations analysis, but also to aid visit-to-visit re-treatment decisions. Alexander et al, studying visit-to-visit change, albeit not with an 'area of interest' guided MP technique, and demonstrated that change in RS may precede change in VA or OCT measures. (Alexander et al, 2012). The principle disadvantage of using RS is that data acquisition takes 8-12 minutes per eye, which is

difficult for frail individuals to achieve and would necessitate substantial resources.

8.2.4.2 Fixation stability

Fixation is impaired in late stage AMD (Pearce et al, 2011). Fixation stability is potentially an important measure of visual function as it correlates with day-to-day tasks such as reading (Ergun et al, 2003; Crossland et al, 2005).

In parallel with the MP assessment of RS, fixation stability data was gathered as an exploratory outcome. It was analysed using the method described by Fujii (Fujii et al, 2003) but in hindsight using the bivariate contour ellipse area, as described by Crossland may have proved more sensitive to detect change (Crossland, et al, 2005). Five of the 8 participants demonstrated no change in the fixation stability (of these 2 already had stable vision), 2 had an improvement in their fixation and 1 showed a decline. These results are broadly in line with other larger published datasets although study designs were not directly comparable (Pearce et al 2011; Munk et al 2013). The relatively small numbers of participants, short study duration and the analysis method precluded meaningful correlation analysis with VA, OCT and RS. Capturing FS data could be useful in determining the preferred retinal location and, in combination with qualitative aspects of OCT such as the presence of retinal fluid, can inform if retreatment is appropriate.

8.2.5 Functional MRI and the BOLD response

This study looked at cortical response to retinal treatment for nvAMD. Data from 10 participants from the same cohort as in the MP study undergoing routine, NICE approved NHS treatment were analysed. All had VA, CRT and fMRI assessments prior to their first treatment and then on a further three occasions, prior to re-treatment. The Blood Oxygenation Level Dependent (BOLD) response from predefined Regions of Interest (ROI) of the visual

cortex, which corresponded either to the macular or peripheral retina, were studied. Averaging of two scans in a session and averaging across hemispheres corrected data for the anticipated normal variation.

The VA improved and CRT decreased broadly in line with larger data sets (Martin et al, 2011, Chakravarty et al, 2012, Schmidt-Urfurth, 2014). There was a decline in BOLD across the four sessions however, in both mean treated and untreated eyes This is a well-documented phenomenon, thought to be due to habituation or attenuation, possibly because the same stimulus is presented at each session (Rosengarth et al, 2013). This decline was accounted for by correcting the treated eye response by the untreated eye response and subsequently the mean treated / untreated BOLD response increased across the 4 sessions. Correlation with VA and CRT both missed significance and this may be explained by under powering of the study or the technique itself.

The main conclusions of this aspect of this study were two fold. Primarily it demonstrated that reactivation of the visual cortex is possible after a period of inactivity due to nvAMD. This confirmed the findings from the single case reported by Baseler et al (Baseler et al, 2012). It was not possible however, to establish to what extent the visual cortex is able to recover, if this was limited by retinal function recovery or indeed if there is a critical window where recovery is possible. Different study designs would be required to address these issues. Secondarily, the range of BOLD response was so great that even if fMRI was practical to perform routinely it would not be a useful tool for measurement of activity from visit-to-visit and therefore to guide retreatment. It may have potential in the clinical setting as a tool to investigate if the cause of 'non response' to treatment is cortical or retinal.

8.2.6 Measuring the side effects of treatment. The amelioration of short-term intraocular pressure fluctuations with oral acetazolamide

Chapter 7 appreciated that outcomes of nvAMD treatment are not always positive (Brown et al, 2006; Rosenfeld et al, 2006) and that thought needs to be give as to how side effects are measured. Side effects with anti-VEGF therapy can be classified as either systemic or ocular and the transient rise in intraocular pressure (IOP) at the time of and after an intravitreal treatment is a well-documented example of the latter. (Falkenstein et al, 2007; Kim et al, 2008). Individuals with glaucoma, who are already at risk of peripheral visual loss due to raised pressure, were studied. The study in chapter 7 was a randomized controlled trial aimed to establish if oral acetazolamide, a diuretic, was able to reduce pressure rise following intravitreal injection in a group of glaucoma and glaucoma suspect patients having treatment for nvAMD.

Of the 24 participants studied, 12 were randomized to receive treatment. This group showed a numerical reduction in peak IOP measurement (42.2mmHg, SD 10.2) compared with the control group (44.3 mmHg, SD 19.8). All points out to the end of the study at 30 minutes demonstrated similar numerical difference and using ANOVA testing no difference between the groups was identified. At the 30-minute single time point measure a difference in the two groups did reach statistical significance (15.7mmHg, SD 4.3, versus 20.6 mmHg, SD 9.5, $p = 0.013$). This, along with the area under the curve post analysis, provides useful information in designing future studies. Despite this statistical difference there is unlikely to be clinical significance in such a small difference in all except those that are extremely vulnerable to an IOP spike.

Discussion followed as to whether it is the IOP that is important to measure or in fact the visual loss caused by the raised pressure that should be the outcome of interest. As progression of visual fields is very slow and can sometimes be unreliable, a study of much longer duration with numerous

intravitreal injections and multiple visual field analyses would be required to address the issue (Choy, Kwun, Han & Kee, 2015). Given the time and resource available, studying the IOP change as a proxy of potential visual change was appropriate.

8.3 Outcome measures of neovascular Macular Degeneration not addressed in this thesis

Outcomes of any treatment can be broadly divided into the following categories: clinical (e.g. efficacy, side effects), humanistic (e.g. role performance, emotional status) and economical (e.g. expenses, saving) (Deshpande et al, 2011). This thesis has mainly addressed clinical and some humanistic outcomes and has not aimed to address economic outcomes. The main focus has been on VA, CRT and IOP as commonly used outcomes and PROMS, MP and fMRI in what could be considered as primarily research tools. Other ways of assessing vision are the topic of the following discussion.

8.3.1 Near (reading) visual acuity and reading speed

The effect on reading performance (reading visual acuity and speed) has been studied in depth in AMD (Legge et al, 1997; Stifter, Sacu, Benesch & Weghaupt, 2005; Richter-Mueksch, Stur, Stifter & Radner, 2006; Cacho, Dickinson, Smith & Harper, 2010). Reading performance has been shown to be significantly impaired in AMD with 60% of the impairment in reading speed being due to reduced near acuity and scotomas (Cacho et al, 2010). This is in contrast to distance acuity, which only has a 10% influence on reading performance (Legge et al, 1992). Late stage AMD disproportionately affects reading performance more than distance visual acuity (Richter-Mueksch et al, 2006). Furthermore reading acuity has been shown to improve more than distance VA when nvAMD is treated (Frennesson, Nilsson, Peebo & Nilsson, 2010).

8.4.2. Contrast sensitivity

Contrast is the luminance or colour of an object that makes its distinguishable from its background. Contrast sensitivity (CS) is the ability to discern the difference in luminance between objects. It has also been well studied in AMD (Mones & Rubin, 2005; Bansback et al, 2007; Patel et al, 2011). In a study of 209 patients with AMD, CS correlated more closely with visual function and quality of life utility than did distance visual acuity (Bansback et al, 2007). Contrast sensitivity was reported to be a more sensitive test of visual function change than distance VA when 121 nvAMD patients were treated with bevacizumab in the ABC trial (Patel et al, 2011). Furthermore OCT morphology correlated well with CS, in particular CS decreasing with an increasing amount of subretinal tissue (Keane et al, 2010). It may be expected that CS would correlate well with RS, however in a small study of 23 participants no relationship was identified. The authors feel that this may be because the study was underpowered (Hautamaki, Oikkonen, Onkamo & Immonen, 2014).

8.4 Conclusions

There are numerous ways of assessing outcomes of the treatment of nvMD. Ultimately the goal of any therapeutics is to maintain or improve quality of life and this can be measured directly with health utility scores. Given their nature, such indicators would perform poorly in assessing the effects of immediate treatment are so best used to retrospectively assess interventions. To assess immediate effect proxy measurements of quality of life are required. It is clear that this principal proxy outcome measure should be governed by the reason for measuring the change. The reasons include the need to describe the response of a population, in which case VA or CRT are appropriate, or to help guide treatment decision, in which case OCT morphology or RS are better suited. Even within these categories some measures may outperform others. For example CRT is a more objective measure than VA, but in some individuals may be of less relevance than VA.

Resources and practical issues are important to consider; the time and ability to perform complex technology dependent measures, even though they are very sensitive, may render them better as research tools. An example of this is the use of fMRI.

The acquisition of many outcome measures can enable a composite view of response to treatment. In this way the individual outcome measurements can work synergistically. This process requires an individual to assimilate all the pieces of relevant information and put them into context of the question to be addressed.

There are many aspects of this thesis that have provided insight into the successful delivery of high quality research; the importance of study design, methodology, attention to the recruitment window and a focus on participant retention have been particularly pertinent. Taking this into account and as is often the case in research, accepting that there are many new questions that have been generated, this thesis has generated useful insights in assessing the outcomes of nvMD treatment.

Understandably there is a tendency for early phase clinical trials to focus on easily quantifiable measure of success such as VA or retinal morphology. I would hope that earlier phases of evaluation of therapeutic interventions would also explore alternative outcomes such as retinal sensitivity. As clinical trials begin to develop for treatment of conditions where the natural history is for visual acuity to change very slowly, such as Geographic Atrophy, clearly new study endpoints are required. Again, retinal sensitivity may be more appropriate. Visual cortex outcomes may also be appropriate in these circumstances, particularly in the knowledge that there may be a loss of volume or activity of the cortex in the long term and that putatively neuroprotection could play an important role. Ultimately, and in particular for the later phase clinical trials, quality of life utilities, which are the most important outcomes for individuals, should be given higher priority.

Appendices

Appendix A. The W-BQ12 Well-being questionnaire

Item	Negative well-being	Energy	Positive well-being
I have episodes of crying or wanting to cry			
I feel down hearted and sad			
I feel fear for no reason			
I become easily upset and panic			
I feel energetic, active and full of vitality			
I feel without energy and weak			
I feel tired, worn out or exhausted			
I wake up felling fresh and rested			
I am happy, satisfied or content with my personal life			
I have the type of life I wanted			
I feel enthusiastic to get on with daily tasks or take new decisions			
I feel I can easily deal with any serious problem or big change in my life.			

Appendix B. The MacTSQ Treatment satisfaction questionnaire

1. How satisfied are you with the treatment for your MD?

2. How bothered are you by any side effects or other effects you experienced with the treatment for your MD?

3. How bothered are you by any discomfort or pain from the treatment for your MD?

4. How do you feel the treatment for your MD is working?

5. How unpleasant did you find the treatment for your MD?

6. How apprehensive did you feel about your most recent treatment for MD?

7. How satisfied are you with any cost to you associated with treatment for your MD?

8. How satisfied are you with the safety of the treatment for MD?

9. Were you given information about your MD treatment, e.g. information about procedures, benefits and any risks? 9a. Was the information you were given in a form you could take home (e.g. in a leaflet)? (b. If yes, was the information given to you long enough before your treatment to allow you to make best use of it? 9c. How satisfied are you with the information provided about the treatment for your MD?

10. If further treatment for your MD were necessary, how satisfied would you be to continue or repeat the treatment?

11. How satisfied are you with the time spent at the clinics on each treatment day?

12. How satisfied are you with the overall duration of the treatment for your MD?

13. Would you encourage someone else with MD like yours to have your kind of treatment?

14. Are there any other aspects of the treatment for your MD, causing satisfaction of dissatisfaction, that have not been covered already?

The MacTSQ can be used as a single scale or as in our study as 2 subscales. Subscale 1 (Information provision and convenience) contains six items (1, 9c and 10 to 13) and subscale 2 (Impact of treatment), which contains six items (2 to 6 and 8). Each question scores between 0 and 6 giving a total possible score of 72.

Appendix C. Raw BOLD data for participants 1-10- Left and right hemisphere projected amplitude across all 4 sessions for both the treated and untreated eye.

	PP_Left Hem_ProjAmp							
	Treated Eye				Untreated Eye			
Participant	Session 1	Session 2	Session 3	Session 4	Session 1	Session 2	Session 3	Session 4
1	0.199852		0.177254	0.028077	0.445891		0.541926	0.120751
2	1.587112	1.142707		1.970797	1.462461	1.433796		1.634187
3	0.197924	0.191190	0.273981	0.211132	0.241968	0.299090	0.146287	0.362546
4	0.300632	0.340123	0.168455		0.437856	0.498992	0.510980	
5	0.035295		0.144161	0.102315	0.121328		0.105470	0.179376
6	0.584713	0.909950	0.388482	0.574210	0.187114	0.213241	0.138982	0.490987
7	0.648770	0.382172	0.656346	0.745081	0.706105	0.370161	0.509113	0.307332
8	0.015962	0.026680	0.166332	0.250102	0.298358	0.145402	0.212310	0.283924
9	0.154021	0.394266	0.183401		0.344309	0.738739	0.528823	
10	-0.456334	0.053861	1.143094	1.456971	0.615644	0.708574	1.150516	1.198214

PP_Right_Hem_ProjAmp							
Treated Eye				Untreated Eye			
Session 1	Session 2	Session 3	Session 4	Session 1	Session 2	Session 3	Session 4
0.190795		0.245601	0.098371	0.276177		0.331438	0.20571
0.595263	0.889731		0.738290	0.266001	1.097948		1.099594
0.299272	0.196710	0.20282	0.375874	0.313938	0.032305	0.422897	0.157396
0.179509	0.061465	0.15052		0.124951	0.150538	0.010907	
0.233128		-0.05200	0.222557	0.556975		0.373369	0.329240
2.710465	2.314634	1.882082	2.126247	1.062131	1.874393	1.599101	2.504829
0.386992	0.252248	0.377487	0.388136	0.506045	0.344709	0.340193	0.276683
0.276350	0.178966	0.294250	0.209231	0.632990	0.220506	0.236842	0.271296
0.760945	1.288191	1.241026		0.871843	1.430766	1.389128	
0.203453	0.006687	-0.36734	0.003686	0.042165	0.066416	0.121872	0.010442

	MC_Left_Hem_ProjAmp							
	Treated Eye				Untreated Eye			
Particpant	Session 1	Session 2	Session 3	Session 4	Session 1	Session 2	Session 3	Session 4
1	0.368023		0.156996	0.123644	0.419915		0.234552	0.002558
2	0.768052	0.828275		1.053571	0.868828	0.767719		0.854110
3	0.264387	0.288367	0.347817	0.226581	0.310856	0.454403	0.270657	0.372601
4	0.212357	0.324846	0.223399		0.064743	0.458877	0.124197	
5	0.859819		0.649731	0.763742	1.046257		1.074632	0.251409
6	0.828071	0.980123	0.806527	0.789223	0.560860	0.598943	0.483433	0.863680
7	0.563859	0.458631	0.713748	0.662989	0.581278	0.367953	0.434152	0.364377
8	0.332584	0.384538	0.238464	0.582487	0.229772	0.573932	0.290741	0.479894
9	0.811453	1.157065	1.450169		1.224781	1.161217	1.184117	
10	0.646467	0.263102	0.310145	0.317467	0.349314	0.330606	0.267022	0.338613

Line_Right_Hem_ProjAmp							
Treated Eye				Untreated Eye			
Session 1	Session 2	Session 3	Session 4	Session 1	Session 2	Session 3	Session 4
0.890321		0.880818	0.276000	0.601474		0.947429	0.200337
0.945582	1.299540		1.030788	0.556175	1.353398		1.340461
0.448729	0.236317	0.354217	0.472443	0.275876	0.268415	0.473296	0.278034
0.603537	0.846508	0.415649		0.398622	-1.123799	0.495722	
0.270391		-0.083958	0.099623	0.744949		0.592507	-0.504903
1.265250	0.897362	-1.127648	1.018246	0.418475	1.322228	0.745803	1.351727
0.656800	0.411092	-0.611596	-0.614306	-0.654271	0.393036	0.499581	0.452691
0.543412	0.693411	0.489000	0.512592	-0.696131	0.636431	0.569816	0.607865
-1.032286	1.796397	1.696452		1.227540	1.696585	1.694391	
0.328651	0.120536	0.037389	0.080819	0.358883	0.269647	0.138510	0.150211

Definitions

ABC	Bevacizumab for Neovascular Age-related Macular Degeneration trial
ANCHOR	Anti-VEGF antibody for treatment of predominantly classic choroidal neovascularisation in AMD study
ANOVA	Analysis of Variance
Anti-VEGF	Anti-Vascular endothelial Growth Factor
AMD	Age-related Macular Degeneration
AREDS	Age-related Eye Disease Study Group
BCVA	Best Corrected Visual Acuity
BH	Base Hospital
BOLD	Blood Oxygen Level Dependent
BSE	Better seeing eye
CATT	Comparison of anti-VEGF Treatment Trial
CEC	Community Eye Clinic
CRT	Central Retinal Thickness
CNV	Choroidal Neovascular Membrane
dB	Decibels
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
FFA	Fundus Fluorescein Angiography
fMRI	Functional Magnetic Resonance Imaging
GA	Geographic Atrophy
ICG	Indocyanine angiography
IOP	Intraocular Pressure
IVAN	Investigation of anti-VEGF Agents in Neovascular AMD Trial
LGN	Lateral Geniculate Nuclei
LogMAR	Logarithm of Minimum Angle of Resolution
MARINA	Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD
MacTSQ	Macular Treatment Satisfaction Questionnaire
MD	Macular Degeneration

MMD	Myopic Macular Degeneration
MP	Microperimetry
MPS	Macular Photocoagulation Study
MRI	Magnetic Resonance Imaging
NEI VFQ	National Eye Institute Visual Function Questionnaire
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
OCT	Optical Coherence Tomography
nvAMD	Neovascular Age-related Macular Degeneration
nvMD	Neovascular Macular Degeneration
PCV	Polypoidal Choroidal Vasculopathy
PDT	Photodynamic Therapy
PED	Pigment Epithelial Detachment
PlGF	Placental Derived Growth Factor
PM	Pathological Myopia
prn	*Pro re nata* / as required
PRO	Patient Reported Outcome
PROM	Patient Reported Outcome Measure
RADINACE	A randomized controlled study of ranibizumab in patients with choroidal neovascularisation secondary to pathological myopia.
RAP	Retinal Angiomatous Proliferation
RPE	Retinal Pigment Epithelium
SAE	Serious Adverse Events
SAILOR	Safety of ranibizumab in subjects with nvAMD trial
SmPC	Summary of Product Characteristics
SPSS	Statistical Package for the Social Sciences
SD	Standard Deviation
TAP	Treatment of Age-related Macular degeneration with Photodynamic therapy study
TAG	Technology appraisal guidance
TTT	Transpupillary Thermotherapy
V1	Primary Visual Cortex

V2	Secondary Visual cortex
V3	Tertiary Visual Cortex
V4	Quaternary Visual Cortex
V5	Fifth Visual Cortex
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor
VIP	Verteporfin in Pathological Myopia study
W-BQ12	12 Item Well-Being Questionnaire
WSE	Worst seeing eye

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