UNIVERSITY OF NOTTINGHAM

An Investigation of the Epidemiology of Age-Related Maculopathy in the UK.

By Craig Wilde
MBChB MRCS FRCOphth

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Contents

Chapter 1 Literature review 1

1.1 General introduction 1

1.2 Study Aims 3

1.3 Introduction 4

1.4 Definitions 5

1.5 Reticular Pseudodrusen 6

1.6 Diagnostic Tests/Investigations in AMD 15

Chapter 2: Methods 24

2.1 Overview 24

2.2 The Bridlington Eye Assessment Project (BEAP) 24

2.2.1 Inception and organization of the Project 24

2.2.2 Eligibility Criteria 25

2.2.3 BEAP Examination Protocol 28

2.2.4 Grading Training 29

2.3 Grading of retinal images 31

2.3.1 Background 31

2.3.2 Photography and Film Processing 32

2.3.3 Grading 35

2.3.4 Definitions for grading 36

2.4 STROBE Statement 41

2.5 BEAP Study Bias 42

2.5.1 Selection Bias 42

2.5.2 Volunteer bias 44

2.5.3 Information Bias 45

2.5.4 Confounding 45

2.5.5 Outcome misclassification 46

2.6 Sample Size 46

2.6.1 Sample size formula for cross sectional studies 47

Chapter 3: Prevalence of AMD in an elderly UK population-an overview 48

3.1 Introduction 48

3.2 Methods 52

3.2.1 Study Design 52

3.2.2 Photographic grading 54

3.3 Results 57

3.4 Discussion 63

Chapter 4: Characteristics of Geographic atrophy in an elderly UK population and the association with reticular pesudodrusen-The BEAP 79

4.1 Introduction 79

4.2 Methods 83

4.3 Results 85

4.4 Discussion 92

Chapter 5: Prevalence of RPD in an elderly UK population 109

5.1 Introduction 109

5.2 Methods 112

5.3 Results 114

5.4 Discussion 120

Chapter 6: Prevalence of peripapillary choroidal neovascular membranes (PPCNV) in an elderly UK population 132

6.1 Introduction 132

6.2 Methods 133

6.3 Results 135

6.4 Discussion 141

Chapter 7: The diagnostic accuracy of SD-OCT vs FFA 153

7.1 Introduction 153

7.2 Materials and Methods 155

7.2.1 Definitions of SD-OCT grading with illustrated examples 159

7.2.2 Sample size calculation 162

7.3 Results 164

7.4 Discussion 165

Chapter 8: False positive diagnosis using spectral domain optical coherence tomography for neovascular age-related macular degeneration: an exploratory study 170

8.1 Introduction 170

8.2 Materials and Methods 171

8.3 Results 173

8.4 Discussion 178

Chapter 9: Prevalence of reticular pseudodrusen in newly presenting adult onset foveomacular vitelliform dystrophy 185

9.1 Introduction 185

9.2 Materials and Methods 188

9.3 Results 191

3 Discussion 193

Chapter 10: Prevalence of reticular pseudodrusen in eyes with newly presenting neovascular age-related macular degeneration 201

10.1 Introduction 201

10.2 Methods 203

10.3 Results 206

10.4 Discussion 208

Chapter 11: General Discussions and Conclusions 216

11.1 ARM and AMD Prevalence in the UK 216

11.2 Prevalence and characteristics of GA in the UK. 217

11.3 Reticular pseudodrusen 218

11.4 Peripapillary choroidal neovascular membranes. 220

11.5 Diagnostic accuracy of SD-OCT for diagnosing nAMD 222

11.6 Causes of false positive diagnosis with SD-OCT for nAMD 223

11.7 Prevalence of RPD in newly presenting nAMD 225

11.8 Future Directions 226

Chapter 12: Appendix 1 STROBE Statement 228

References 237

Figure 1: Chart depicting prevalence of AMD by age-group. 76

Figure 2: Colour fundus photograph of typical horseshoe shaped GA with foveal preservation. 106

Figure 3: Colour fundus photograph showing small multifocal GA. 107

Figure 4: Colour fundus photograph of reticular pseudodrusen with horseshoe shaped pigmentary change 108

Figure 5: RPD associated with FP features that can be suggestive of ACA. 129

Figure 6: RPD with central subfield involvement. 129

Figure 7: U shaped foveal sparring GA, with clearly visible superotemporal RPD. 130

Figure 8: Topographical distribution of prevalent RPD for the right eye in the BEAP. 130

Figure 9: Topographical distribution of prevalent RPD for the left eye in the BEAP... 131

Figure 10: An example of a PPCNV, with visible membrane and exudation. There are visible RPD..... 134

Figure 11: SD-OCT example of subretinal choroidal neovascular membrane..... 159

Figure 12: An example of the SD-OCT features of a fibrovascular PED..... 160

Figure 13: SD-OCT of a drusenoid PED with subretinal space 160

Figure 14: SD-OCT features of a peripapillary CNV..... 161

Figure 15: SD-OCT of disruption of the retinal pigment epithelium with presumed intraretinal fluid 161

Figure 16: An example of AFVD associated with RPD using the Heidelberg SD-OCT. . 197

Figure 17: Heidelberg SD-OCT demonstrating discrete hyper-reflective collections in the subretinal space..... 199

Figure 18: A colour fundus photograph demonstrating reticular pseudodrusen..... 199

Figure 19 :Topographical representation of the fundus distribution of RPD in eyes with AFVD. 200

Figure 20: A colour fundus photograph demonstrating reticular pseudodrusen. There are multiple round and oval yellow-white lesions that join to form an ill -defined network of broad, interlacing ribbons..... 214

Figure 21: Enhanced visualization of RPD with digital enhancement of photograph to red free..... 214

Figure 22: SD-OCT demonstrating discrete hyper-reflective collections in the subretinal space giving a saw-tooth appearance. 215

Table 1: Modified Rotterdam AMD Grading Scale 69

Table 2: BEAP AMD grading results for right eye, left eye and worse eye. 70

Table 3: Sex distribution of worse eye Rotterdam AMD grade. 71
Table 4: Age distribution by worse eye Rotterdam AMD score. 72
Table 5: Mean age distribution of Rotterdam grades. 73
Table 6: Subject self-perception of vision as being satisfactory, with corresponding AMD grades. 74
Table 7: Odds ratios for dissatisfaction with vision when compared to subjects with grade 0a/0b 75
Table 8: Summary of spectacle corrected visual acuity (SCVA) for right and left eyes across AMD grades, subcategorised for level of visual impairment. 77
Table 9: A comparison between reported prevalence of AMD grades (in either eye), across age-groups and gender. 78
Table 10: GA prevalence for right eye, left eye, worse eye and best eye. 89
Table 11: Age specific prevalence of GA for right eyes and left eyes and worse eyes. . 89
Table 12: Summary of GA parameters for right and left eyes. 90
Table 13: Contingency table comparing subfoveal GA vs extrafoveal GA for eyes with and without reticular pseudodrusen. 91
Table 14: A contingency table comparing multifocal GA vs solitary GA for eyes with and without reticular pseudodrusen. 91
Table 15: Contingency table comparing U-shaped/horseshoe GA with round GA for eyes with and without reticular pseudodrusen. 92
Table 16: Gender specific prevalence of reticular pseudodrusen. 117
Table 17: Age specific prevalence of RPD. 117
Table 18: Comorbidity of RPD with conventional drusen, RPE pigmentary changes and AMD. 118
Table 19: An exploration of satisfaction with vision. 119

Table 20: Population prevalence of PPCNV 137

Table 21: Age specific prevalence of PPCNV 137

Table 22: Summary of demographic details of eyes with PPCNV and associated macular age-related changes (Rotterdam Grades), with visual acuity (VA)..... 138

Table 23: Summary of the peripapillary retinal changes along with size (area) and locations of CNV in relation to the optic disc in all the eyes with PPCNV. 139

Table 24: Colour fundus photograph modified Rotterdam grade for AMD stage for all SD-OCT false positive eyes..... 176

Table 25: Correlation between SD-OCT false positive subgroups and FP Rotterdam grades..... 177

Table 26: Association of presumed SRF and IRF as identified using SD-OCT stratified for AMD stage as per Modified Rotterdam grade and SD-OCT subcategory..... 184

Table 27: Comparison of characteristics in patients with or without reticular pseudodrusen in the ipsilateral eye with newly presenting nAMD..... 212

Table 28: FFA subtypes of neovascular AMD with and without reticular pseudodrusen. 213

Table 29: Contingency table for RAP with and without reticular pseudodrusen. 213

I dedicate this thesis to my father, who sadly passed away many years ago now. His support and encouragement in my early years has been a constant motivation to aim higher, simply do my best and work hard. His gift of time well spent will forever be remembered. I also dedicate this thesis to my mother, whom has been a constant tower of support and strength over the years, making many personal sacrifices for the good of all her children.

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Chapter 1 Literature review

1.1 General introduction

Age-related maculopathy (ARM) is a progressive and degenerative disease of the central area of the retina, the macula. It is often associated with visual impairment in the late stages. It has an increasing prevalence with age and has been described for over 100 years, with the disciform variant being first reported in 1875[1]. It is the leading cause of blindness in developed countries and the third largest cause of global blindness [2, 3]. In its early stages ARM is characterized by the deposition of yellowish deposits below the retinal pigment epithelium (RPE) called drusen, along with RPE pigmentary abnormalities, such as patches of hyperpigmentation or hypopigmentation. These early changes are usually not associated with a loss of vision. There are two main types of late stage disease, which are referred to as age related macular degeneration (AMD). Neovascular AMD (nAMD) is where there is invasion of the sub-retinal and sub-RPE spaces by neovascular complexes originating from the choroid. This process is called choroidal neovascularization (CNV). Geographic atrophy (GA) is characterized by loss of the choriocapillaris and the overlying RPE. Visually impairing nAMD is around twice as prevalent as GA [4]. Together these changes represent the late stage of a number of pathological changes in the retina that occur with aging.

The prevalence of ARM and AMD has been reported across a range of population settings, predominantly in developed countries [5] such as Australia [6, 7], the United States [8] and Europe [9-14]. Prevalence measures as high as 25% in the over 75-years population have been reported [15]. With an increasing demographic shift towards an aging population, the prevalence of AMD is expected to double unless preventative strategies are developed [16, 17]. Of the European studies, all but one was undertaken in northern Europe [9-14]. Three of these European studies were small, with less than 500 participants [9, 12, 14] or included patients with a younger age and so had few cases of AMD [10, 14].

There are several publications on the prevalence of AMD in the UK population. Some are not community based, while others report measures of prevalence based on data from registration for visual impairment or using meta-analysis data from UK studies and elsewhere [4, 14, 17-19]. One major problem with their methods is that data from registration of visual impairment may be incomplete or inaccurate. Furthermore, such investigations only include visual impairment associated with late AMD. While patients with bilateral visual impairment may be registered as visually impaired, individuals with unilateral, asymmetric or eccentric disease, with preservation of good visual acuity may go unrecorded. As a consequence the current UK prevalence estimates do not include patients will early, asymptomatic ARM and there is inadequate data on the asymmetry of AMD. Individuals with advanced AMD in one eye and near normal visual acuity in the other may go unrecorded. There are few UK population based prevalence studies with large sample sizes where the available data is not dated

[12, 20, 21]. A recent study by Ngai et al (2003) included only male subjects only aged 65-83 years[22]. The European Eye Study (EUREYE) estimated the prevalence across 7 European countries, but only included 634 individuals for its UK center in Belfast, Northern Ireland[23]. The study by Evans et al (2004) restricted the age range of patients to 75 years and above [24].

The reported prevalence of AMD across these studies differs significantly. This may reflect the different assessment criteria and age groups included, but indicate the need for a large study to provide a more accurate estimate of the prevalence of the different stages of AMD in the UK population. In order to address these shortcomings in the current available data, a systematic review was commissioned by the VISION 2020 UK Macular Interest Group to provide estimates of how many people in the UK have AMD, based on previously published data [25, 26]. This review provides reliable estimates for the prevalence/incidence of late AMD. However, it does not provide an accurate measure of the prevalence of the early stages of ARM or individuals with asymptomatic or asymmetric disease in the UK population.

1.2 Study Aims

1. To determine the prevalence of different stages of ARM/AMD in the elderly UK population and their correlation with measured visual acuity and explore associations with systemic risk factors.

2. To report the community prevalence and risk factors of reticular pseudodrusen (RPD) and to determine their morphology and retinal distribution.

3. To determine the prevalence of RPD in patients presenting with new onset AMD in a hospital eye service population will be established.

4. To assess the diagnostic accuracy of SD-OCT with and without colour fundus photography (FP) in the diagnosis of newly presenting exudative AMD in comparison against fundus fluorescein angiography (FFA).

5. To explore the reasons behind false positive errors with the use of SD-OCT in the diagnosis of nAMD and to potentially develop a clinical OCT grading system that could be used to guide diagnosis in patients where FFA is not possible.

6. To provide a measure of prevalence of asymptomatic PPCNV.

7 To provide a detailed measure of the prevalence of GA within the UK and provide information regarding the features of GA including mean area, the prevalence of multifocal GA and proportion of eyes with foveal involvement.

8. To explore the association between RPD and other retinal and macular diseases that may share similar pathophysiological mechanisms.

1.3 Introduction

ARM and AMD are progressive and degenerative disease of the central area of the retina (the macula) that is often associated with visual impairment in the late stages [3]. It has an increasing prevalence with age and has been described

for over 100 years, with the disciform variant being first reported in 1875 [1]. It is the leading cause of blindness in developed countries and the third largest cause of global blindness [2, 3].

1.4 Definitions

Definitions are based on internationally accepted and previously used classification systems [27] .ARM is the term applied to ageing changes, in the absence of any other precipitating cause. It occurs in the central area of the retina in people aged 55 years and above [26]. In the earliest stages of the disease a lipid material accumulates below the RPE and within Bruch's membrane. When focal collections of this material reach a significant volume they can be seen as yellow deposits on clinical examination of the retina and are referred to as drusen. The RPE also undergoes changes that are detectable on fundoscopy as areas of hyper/hypo pigmentation. As a general rule early changes associated with drusen deposition and RPE pigmentary changes are not associated with impairment in central vision. When visual loss does occur it is often secondary to the development of GA or exudative disease.

A Bayesian meta-analysis was performed using data from 31 population based studies, including a total of over 50, 000 individuals, to create prevalence measures for age and gender in people of European ancestry[26]. Using this methodology Owen et al (2012) estimated the age specific prevalence of late AMD, GA and nAMD [25]. The estimated overall prevalence of late AMD was 2.4% (95% credible interval 1.7% to 3.3%), equivalent to 513,000 cases. This was estimated to increase to 679, 000 cases by 2020. Prevalence was estimated to

be 4.8% in the 65 year and over age group, increasing to 12.3% for individuals aged 80 years and over[25]. Although the prevalence may be estimated to increase given an aging population, it is interesting to note that the incidence of legal blindness attributed to AMD may not follow this trend. In Denmark there has been a reduction in the incidence of legal blindness between 2000 and 2010 of 50% [28]. The reduced incidence coinciding with the introduction of anti-vascular endothelial growth factors (anti-VEGF).

1.5 Reticular Pseudodrusen

RPD were first described in 1990 by Mimoun et al as a peculiar yellowish pattern in the fundus of patients with AMD [29]. The structures were named 'les pseudo-drusen visibles en lumiere bleue', because they had enhanced visibility when viewed using blue light. One year later Klein et al in the 'Wisconsin Age-Related Maculopathy Grading System' (WARMGS), classified them as 'pseudodrusen' and considered them similar to soft drusen [27]. Arnold et al [30] subsequently reported they their presence as a yellow interlacing network, ranging from 125-250 μm in size and referred to them as RPD. They also made the first histological report of RPD in one eye, which had lost its retina post mortem. They reported loss of choroidal vessels with replacement of the choroidal stroma with fibrous tissue. They did not reveal any visible correlates with drusen. Since, based on this finding the term pseudodrusen has been used in most studies. The patient did have markedly thin choroid [30] and the histopathological analysis demonstrated a significant loss of the middle choroidal layer of small vessels and increased spacing between large choroid

veins. This led to the hypothesis that the aetiology of RPD may involve fibrotic replacement of choroidal stroma and a loss of choroidal vascularity leading to the development of RPD. It was hypothesized that this could be a marker of choroidal ischaemia [30].

Photographs and descriptions of RPD vary considerably between different publications [30-37] seemingly because of the different diagnostic criteria applied and a lack of standardization. Some studies have enhanced the visualisation of RPD with blue light. None of these publications have defined how this was determined [29, 35]. Classification systems used in major epidemiological studies have varied considerably, with RPD classified in various ways including soft drusen, or a separate entity called reticular soft drusen [35, 36, 38-40]. None of these studies used blue light imaging. One reading center had previously suppressed the blue channel of color photographs [41]. One previous study defined RPD as being better seen with red-free light and not seen well with fluorescein angiography [32]. Another publication did not clearly define their diagnostic criteria [42]. In the WARMGS they were described as reticular drusen and defined as an 'ill-defined networks of broad interlacing ribbons'[27]. RPD were not specifically recognized in the International Classification and Grading System for AMD[43].

Zweifel et al (2012) used spectral-domain optical coherence tomography (SD-OCT) to demonstrate discrete collections of hyper-reflective material, located not under (as typical drusen are) but above the RPE [44]. They localised these lesions below the inner segment/outer segment junction and named them sub-

retinal drusenoid deposits (SDD). On color fundus photography this material could have an appearance similar to soft drusen, but with SD-OCT the material was reportedly clearly differentiated. When these SDD were co-localised using either near infrared or when examining the blue channel of color fundus photography with the abnormalities seen on SD-OCT, many more SDD were visualized using SD-OCT. The histological composition of SDD has been found to be similar to that of soft drusen [45]. Both have membranous debris, cholesterol esters, cholesterol and positive staining for complement [16, 44]. For this reason some authors prefer the term SDD as they feel it is a more descriptive term than RPD, which is vague and has various past definitions.

Indocyanine green angiography (ICGA) represents another method that has been used for the in vivo analysis of RPD. It was introduced as an imaging modality of the choroid in the 1970's because of the particular optical properties of the dye [46]. Indocyanine green (ICG) absorbs and reflects in the near infrared position of the spectrum (805 and 835nm respectively) and thus the RPE is essentially rendered invisible. In 2012 Querques et al reported that large choroidal vessels as visualized with ICGA co-localised to areas of iso/hypereflectivety on infra-red (IR) reflectance imaging [47]. Individual RPD lesions appeared as areas of hyporeflectivity using IR reflectance imaging and were closely abutting but not overlying the large choroidal vessels [47]. The authors reported that these findings are consistent with the impaired choroidal filling that has been reported in eyes with RPD [29, 30, 34] and explains why the extent of SDD on SD-OCT cannot account for the extent of reticular patterns

seen with photography. Sohrab et al (2011) have also reported similar findings [48]. By analyzing en face sections of SD-OCT scans, they localised the reticular pattern to the intervascular choroidal stroma; suggesting that the SDD (which did not co-localise with the reticular pattern) may represent secondary mechanical or biological disturbances to the overlying RPE and outer retina [48]. Querques et al (2012) also demonstrated, using enhanced depth imaging OCT, that the subfoveal choroidal thickness was reduced in eyes with only RPD (without medium/large drusen), compared to eyes with early AMD (defined as the presence of 5 or more medium sized drusen 63-124 microns) or any large drusen over 125 microns within the macula without EPD [47]. The group also found the choroidal thickness of eyes with RPD to be thinner than that of eyes with early AMD at all measurement points except 3000 micrometers superior to the fovea[47]. Interestingly RPD were localised mainly in the superior macula, corresponding to the area of slight choroidal thickening. Querques et al (2012) suggested that when these in vivo SD-OCT findings are taken together with the histological findings of Arnold et al [30], the data suggests that RPD development may be associated with a diffuse loss of small choroidal vessels (and a choroidal thinning), and later a fibrotic replacement (and thus thinning thickening) mainly in the area of higher concentration of RPD. It was hypothesized that derangement of the RPE resulting from atrophy and fibrosis of the choroid could lead to the accumulation of photoreceptor outer segments above the RPE, creating SDD [47].

Zweifel et al (2012) suggested that the hyperreflective material above the RPE could be graded by its thickness and proposed a grading system of 3 stages[44]. Stage 1 is characterized by a diffuse deposition of granular hyperreflective material between the RPE and the boundary between the inner segments (IS) and outer segments (OS) of the photoreceptors (IS/OS boundary). Stage 2 is graded when mounds of accumulated sub-retinal material are sufficient to alter the contour of the IS/OS boundary. In stage 3 the material has a conical appearance, is thicker and breaks through the IS/OS boundary. Querques et al (2012) used this grading system to analyse the morphological changes of RPD over a 24-month period using the eye-tracker follow-up protocol of the Spectralis SD-OCT [49]. They graded stage 4 (a modification from the previous classification by Zweifel et al; 2012) RPD as the last stage of evolution, which was defined as fading of the material because of reabsorption and eventual migration within the retinal layers. They analysed 48 eyes of 33 consecutive patients with RPD. A total of 78 SDD showed progression over a mean time of 23.9 months. All 58 SDD (100%) graded as stage 1 at baseline progressed to stage 2. 13 of 16 SDD's (81.3%) graded as stage 2 at baseline examination progressed to stage 3 and 3 (18.7%) progressed to stage 4. All 4 SDD that were graded as stage 3 at baseline progressed to stage 4. Despite the limitations of this study, such as the potential inaccuracies with the placement of follow up scans, the findings suggest that RPD are a dynamic pathological structure, whose progression on SD-OCT is characterized first by accumulation of focal

material above the RPE and later by reabsorption and eventually potential migration of the material within the inner retinal layers[49].

Previously assumed anatomical attributions of hyperreflective bands on OCT imaging have been demonstrated not to be correct [50]. The presumed IS/OS junction has subsequently been renamed the ellipsoid zone and is now believed to represent mainly mitochondria within the ellipsoid layer of the outer portion of the inner segments of the photoreceptors [50, 51].

RPD have now been described by many research groups and have been shown to be associated with a high risk of progression to neovascular AMD [29-32, 34, 37, 52]. Most of these observations have been from case reports, case series, or clinical trials. In 2008 Klein et al [37] using standardized procedures for obtaining stereoscopic color fundus photographs of the macula and an objective grading system, found a prevalence of RPD of 0.7%, and a 15-year cumulative incidence of 3.0% in the Beaver Dam population aged 43-86 years at baseline. Of the 32 people at baseline with RPD, 20 (63%) had bilateral involvement. When present, they were found outside the macular grid area 81% of the time. When present within the macular grid they were most commonly located within the outer superior subfield (74% of the time), followed by the outer temporal subfield (70% of the time). They were never located within the central subfield [37]. These rates within each location were not mutually exclusive. The reasons why prevalent and incident RPD are more likely to be found in the outer superior and temporal location of the macula may reflect differences in choroidal blood

flow (more interconnections of the choriocapillaris nasally) or could possibly relate to how light or UV exposure affects different parts of the retina [53, 54].

There was an increase in both the prevalence and incidence of RPD with increasing age [37]. No eyes were identified where RPD disappeared without the appearance of lesions such as RPE depigmentation or AMD. This is distinctively different from soft drusen where approximately 20% of soft distinct and indistinct drusen regress in the absence of progression [38], although some of these may be accounted for by initial erroneous grading.

Klein et al reported that eyes with RPD were more likely than eyes with other types of soft drusen to progress to late AMD, with a15-year cumulative incidence of late AMD of 43% and 46% for right and left eyes respectively [37]. The incidence for both pure GA and exudative AMD in the Beaver Dam population was similar. The incidence of visual impairment (the development of a BCVA of 6/12 or worse in the better eye in patients with RPD at baseline over the 15-year period) was 58% and 75% in the right and left eyes respectively. Patients who had RPD at baseline had a 54% poorer survival [37]. This relationship to poorer survival, independent of systemic risk factors, unlike typical drusen, may be a marker of a pathophysiological process associated with death that was not measured in the Beaver Dam Eye Study (BDES) [37]. Interestingly RPD have consistently been found to be a more prevalent finding in woman and also older subjects [30, 37, 55]. This is surprising given that conventional drusen and pigmentary changes do not appear to have different gender specific prevalence rates. As RPD are known to be strongly associated with both GA and nAMD, it

raises the question of why this increased female preponderance of RPD is not translated into higher gender specific prevalence rates of AMD. One possible explanation for the gender disparity may therefore be that RMD is associated with a systemic pathology or risk factors that confer increased mortality primarily among male subjects, whom subsequently die or are too ill to attend population or hospital based studies. The Framingham study demonstrated that men aged 35 to 84 years have twice the incidence of morbidity and mortality due to coronary artery disease compared to woman [56]. It could therefore be hypothesised that cardiovascular pathology and premature death in males may be the cause for the female preponderance of RPD.

In the BDES, Klein et al reported a 54% decreased survival rate in subjects with RPD at baseline [37]. There is increasing evidence to suggest an association between RPD and cardiovascular problems. Boddu et al [57] reports patients with RPD are more likely to be hypertensive than those with large soft drusen. In the Melbourne Collaborative Cohort Study, Finger et al found that RPD were associated with a moderately elevated systolic blood pressure, current smoking and a trend for an association with a history of myocardial infarction or stroke [58]. The association was however reported to disappear in their multivariate analysis, indicating a modest effect size. In the BDES, while controlling for age, RPD were associated with being female (OR 2.67, 95% CI 1.16, 6.17, p=0.02), lower income (OR per lower income group 1.75, 95% CI 1.16, 2.62, p=0.007), body Mass index (OR per 1kg/m^2 1.08, 95% CI 1.02, 1.15, p=0.006) and more pack years smoked (OR 35 or more pack years smoked vs none 2.61, 95% CI

1.17, 5.85, p=0.02). In the Blue Mountains Eye Study (BMES), Tan et al found that high-density lipopreoteins (HDL) were inversely related to the incidence of late AMD [59]. A history of any cardiovascular disease, including stroke, myocardial infarction or angina was also associated with incident early AMD and incident soft or reticular drusen [59].

Different phenotypes of GA are being recognized. Some, such as the 'diffuse-trickling' subtype identified with the use of fundus autofluorescence (FAF) imaging [60] [61] appear to be associated with RPD. In their recent paper Fleckenstein et al demonstrate not only the universal finding of RPD in these eyes but report 54% of these individuals had been hospitalized due to cardiovascular disease including hypertensive crisis, angina and myocardial infarction (MI) [61]. They report these patients had a higher rate of MI in the age group younger than 65 years (24% vs 0%), p=0.011), all but one patient in the cohort of 61 who had an MI were male. Interestingly, they found that at first presentation individuals with the diffuse trickling GA phenotype were younger than those with a non-diffuse trickling phenotype (68.2 ±11.6 vs 75.4 ±8.1, p<0.001). There was also a shift in the proportion of men, form 55% in the younger age group under 65 years to 19% in the over 65 year's population. This adds further weight to the suggestion that cardiovascular disease and the premature death of men may explain why there is a female preponderance of RPD in older populations.

Another consistent finding in the literature is that RPD are associated with choroidal thinning [62 47]. Querques et al report an overall thinned choroid in eyes with RPD and Garg and colleagues demonstrated choroid thinning in the macular area of patients with early AMD and RPD when compared to eyes with only early AMD [63]. Arnold et al in the first histological report of one eye with RPD reported loss of choroidal vessels and replacement of choroidal stroma with fibrous tissue that formed a reticular pattern.

GA is a characteristic area or areas of pallor with sharply defined and scalloped edges that generally involves the part of the eye responsible for central, detailed vision. It is often of an insidious onset and currently no effective treatment is available. Exudative AMD most often presents much more acutely, with patients complaining of blurring and distortion of their central vision. Pathologically it is composed of a CNV that grows in various layers of the retina. If left untreated these abnormal blood vessels cause irreversible loss of central vision, through a combination of leakage and bleeding. An effective treatment for exudative AMD currently exists with the use of intravitreal Lucentis, an anti-VEGF. Once the condition is suspected clinically it is very important that a rapid diagnosis is made, allowing the prompt initiation of treatment. Any unnecessary delay in treatment could result in a less favorable visual outcome.

1.6 Diagnostic Tests/Investigations in AMD

FFA is currently the gold standard for the diagnosis of exudative AMD, as recommended by the Royal College of Ophthalmology guidelines for the

diagnosis and treatment of AMD [64]. FFA's have been used routinely within both research and a clinical setting for many years. They have been the gold standard imaging modality for the diagnosis and sub-classification of CNV in many randomised controlled trials.

FFAs, despite their widespread use, are not without disadvantages. Firstly they are an invasive procedure, requiring appropriate written consent prior to them being performed. Serious adverse reactions are extremely rare but they are known to occur [65]. Yannuzzi et al (1986) estimated the risk of death following FA to be 1: 222 000[66]. Minor adverse reactions are not uncommon. Patients have to be cannulated, which can prove difficult in patients with difficult venous access. It is essential that facilities for resuscitation be available. They are time-consuming investigations, taking around 15 minutes to complete. The quality of the images attained is very operator dependent and requires a good deal of skill and experience: a role generally undertaken by specially trained ophthalmic photographers. If an ophthalmic photographer is unexpectedly ill or away from work for a prolonged period there could be a significant delay in diagnostic imaging and initiation of treatment.

It is sometimes difficult to perform FFA's on the day of presentation for a patient with suspected exudative AMD. It often requires forward planning and patients are frequently brought back for separate appointments for a booked FFA. This can be a delaying step in the initiation of a patients' treatment. Other imaging modalities, such as SD-OCT have dramatically gained importance for both the diagnosis and management of AMD. Noninvasive cross-sectional

images of the neurosensory retina and the subretinal space are obtained, allowing a detailed characterization of structural changes. They are now increasingly used to determine both the presence and activity of CNV and the need for treatment/retreatment in clinical trials and routinely in clinic [67-69]. SD-OCT is an imaging modality that is non-invasive. It requires no patient cannulation and there is zero clinical risk involved for patients. It is an investigation that is very quick to perform. The machine is very easy to operate. Training on its operation is very easy.

The role of SD-OCT as a diagnostic tool however, in the setting of a specialist AMD clinic, has not been widely investigated. In an elderly patient with acute or subacute visual loss combined with subretinal macular haemorrhage and SD-OCT imaging demonstrating a fibrovascular pigment epithelial detachment, some would argue that a diagnosis of nAMD could be made with confidence. This is particularly the case with SD-OCT, given that focal areas of macular pathology are less likely to be missed with raster scanning of the macula [70]. There are very few clinical studies to date that have evaluated the accuracy of OCT imaging against the reference standard of stereoscopic FFA for the diagnosis or detection of nAMD [71-75]. Across the few studies performed different sensitivities and specificities for the detection of CNV are published. Do et al [73] for instance report a sensitivity of only 40% for the detection of new onset CNV in eyes at high risk of developing the disease. This low specificity may be explained by the use of the Stratus time-domain OCT machine, which was readily available at the initiation of the study. Compared to SD-OCT, these

machines have a less dense array of lower resolution images, with more movement artifacts [54]. Published data suggests that SD-OCT is more sensitive in detecting anatomical signs of CNV activity in eyes that are being managed for CNV compared to time domain OCT [76]. The primary objective of the AMD DOC study[73] was to evaluate the sensitivity of time-domain OCT, relative to FFA, in detecting new onset nAMD within a two-year period. The number of patients involved was small (98 enrolled in the study) and only 13 individuals met the primary definition for conversion; in which the FFA was positive according to the reading center and treatment was recommended by the study investigator. All patients had occult lesions with no classic component. Five had a lesion size of less than one disc area, six had lesions that were between1 and 5 disc areas in size and 1 had a lesion that was less than 9 disc areas. The lesion size for the remaining converter could not be measured. Another contributing factor to the low sensitivity is likely the chosen definition for a positive OCT. An OCT was graded as positive if any of the following observations were met: (1) a 10% increase in the central subfield thickness measurement relative to baseline (equivocal to at least 25micrometre increase), (2) SRF was questioned or graded as definitely present, or (3) intraretinal cystoid abnormalities or interstitial fluid were questioned or definitely present. Grading definitions used will influence the sensitivity directly. Do et al (2012) do not use the presence of a fibrovascular pigment epithelial detachment (FV-PED) within their definition of a positive OCT [73]. This could in part contribute towards a low sensitivity. It could be argued that the comparison between the two diagnostic imaging modalities

is flawed secondary to omissions of this nature in the grading process, making a like for like comparison difficult.

Growth of a CNV in the sub-RPE space can produce an irregularly elevated RPE visible clinically and on OCT. This is termed a FV-PED. On fluorescein angiography a FV-PED can be associated with an area of stippled or granular hyperfluorescence that appears in the late frames[77]. On OCT, PED's appear as broad elevations of the RPE band relative to Bruch's membrane [78] and these correlate with an occult lesion on FFA. FV-PED's may be accompanied by variable amounts of serous exudation and/or haemorrhage. As a result, the slope of the PED may vary according to its fluid content, as can the amount of intra/sub-retinal fluid (IRF/SRF) and retinal thickness. By grading an FFA as positive for new onset CNV with the presence of an occult lesion as evidenced by stippled hyperfluorescence but not grading an OCT positive for new CNV with an isolated FV-PED there is likely an inherent bias towards reducing the sensitivity of OCT when comparing these two imaging modalities. Early FV-PED's may not have induced any changes above the RPE and may be clinically static. In this way it is also easy to see why there were four false negatives using OCT imaging; if there are FVPED's with no overlying retinal thickening or SRF, the OCT would have been graded to show the absence of disease. However, the FFA would likely have demonstrated a stippled hyperfluorescence and hence would be graded as positive for an occult CNV. Measuring progression or activity of an occult lesion is different from its diagnosis and recognition. Do et al (2012) may have failed to make this distinction adequately [73].

Sandhu el (2005) al used a comparatively more robust grading classification for OCT images and included OCT features with and without fluid [71]. They also tried to define lesion sub-types by identifying several specific features. Classic CNV was graded with the presence of a subretinal band corresponding to the RPE and choriocapilliaris which is thickened and disrupted, typically giving a fusiform or 'cigar' shape with/without IRF/SRF. Occult CNV was identified using time-domain OCT by a less well defined band than a classic CNV, but appears to be more sub-RPE, with more disorganization of the retina and SRF/IRF (cystoid). A serous PED was graded by the presence of by a dome shaped elevation of the reflective band corresponding to the RPE, with an area of low reflectivity underneath [71]. This study was larger, with 84 cases of CNV identified with FFA (56 classic, 25 occult and 3 serous PED's). The OCT used was a time domain OCT. Six 6mm radial scans were used for the macular scanning protocol and if an abnormality was detected, further cuts were taken by manually moving the scan. Despite the use of time-domain OCT, Sandhu et al (2005) [71] reported a much higher sensitivity (96.4%) than Do et al (2012) [73]. They reported a similar specificity (66%). When OCT grading was performed in conjunction with the use of colour stereoscopic FP, the specificity increased considerably to 89.4%. The use of stereo photographs could be considered to provide similar information to clinical biomicroscopic examination.

With any diagnostic test, the number of patients with a disease, classified as disease free (false negative) is very important. Sandhu et al (2005) in their study missed only 3 cases of CNV that were identified with FFA [71]. They mention that two of these missed cases were diagnosed as having only drusen/atrophy. However, on FFA both had small areas of CNV, which the authors report were likely missed by the cut of the OCT scan. With the much greater sampling density of spectral domain raster scanning, these false negatives may have been avoided. The other missed case was diagnosed as having an epiretinal membrane. On retrospective review the authors noticed the presence of SRF. Again, the use of SD-OCT raster scans may have been more likely to detect the SRF or other lesions in-keeping with CNV.

Sandhu et al (2005) reported a high false positive rate of 34% with the use of time domain OCT alone [71]. This was however reduced to 10.6% with the addition of FP. The authors comment that on retrospective review, two of these false positives were reclassified as non-CNV. The remaining 14 false positives were still felt by the authors to have abnormal OCT images. They did not however go on to report what these features were or give any mention of long term outcome. The context of the Sandhu et al (2005) paper has to be considered. It was a prospective series of images collected over a six-month period for patients who were presenting for the first time for suspected CNV. There were a total of 21 other diagnoses made other than CNV, including 3 retinal vein occlusions, 2 cystoid macular oedema patients, two epiretinal membranes, 2 central serous retinopathies and diabetic retinopathy among

others. The diagnostic criteria are much generalized and it may be possible to develop an OCT grading system that will reduce the number of false positives and negatives further.

Sandhu et al (2012) reported that defining components of CNV using OCT was complex. Late leak occult CNV's were particularly difficult to categorise, appearing to have components of classic CNV, cystoid macular oedema and a PED [71]. One fifth of occult lesions were classified as classic CNV by OCT. The authors report there was also difficulty distinguishing a serous from a vascularized PED, with the OCT given the poor penetration below the RPE. Perhaps with a more detailed grading protocol the ability to identify lesion subtypes could be vastly improved.

A recent publication by Mokwa et al (2013) has reported a sensitivity and specificity of 94% and 98%respectively with the use of SD-OCT for identifying new onset CNV[74]. In their study 68 patients were identified with CNV using FFA. The authors remind us again of the inadequacies with the identification of CNV lesion subtypes using SD-OCT. In their study 5 cases of presumed classic CNV (demonstrating subretinal hyperreflective material) had occult lesions on FFA, without the presence of classic leakage or a staining scar. It highlights the fact that subretinal hyperreflective material on OCT does not only represent a type 2 subretinal membrane, but perhaps subretinal haemorrhage, fibrous material or photoreceptor debris. It emphasizes the need for more robust grading techniques. Perhaps a more systematic grading system and more detailed analysis of the OCT pseudo-histological features may help improve lesion

subtype classification. Another important finding was that a PED was seen in 22 eyes without evidence of occult lesions on FFA. In these cases other CNV lesion components such as overlying scar or a subretinal classic CNV would cover the CNV membrane located in the sub-RPE space. In this way occult lesions can be missed and it highlights a potential error with sole reliance on FFA for CNV classification. Even the use of stereo-FFA may prevent the identification of a shallow FV-PED with an overlying classic with retinal thickening. It is important to remember the potential benefits that high resolution cross sectional OCT imaging may have over FFA.

Chapter 2: Methods

2.1 Overview

The methods involved for the body of work contained within this thesis are divided into two broad groups. The first part of the study forms an exploration of the epidemiology of AMD using a community based population study, The BEAP. Secondly, I utilize a hospital based population to generate cases of AMD to explore the relationship between advanced AMD (and its angiographic subtypes) specifically with RPD. I also explore the association between rarer conditions such as adult onset foveomacular vitelliform dystrophy (AFVMD) and RPD using this population. The methods utilized for the hospital based studies will be discussed within each relevant chapter. Only the methods of the BEAP will be explored in detail here.

2.2 The Bridlington Eye Assessment Project (BEAP)

2.2.1 Inception and organization of the Project

The BEAP was conceived by Professor SA Vernon (consultant Ophthalmologist at Queens Medical Centre, Nottingham) and Doctors JG Hillman and HK McNab (General Practitioners, Bridlington) [79]. The primary purpose was to investigate the utility of screening for eye diseases in an elderly population. It utilised both digital imaging technologies and clinical examination by a trained optometrist. The elderly population in Bridlington is quite stable with little migration. The

community therefore offered an ideal opportunity for a screening project, given the long duration required for completion. The project is a registered charity (charity number 1091980) with a board of Trustees. The project was organized by a project manager and administered by two receptionists. Other staff employed for the purposes of the project included one registered nurse and four local optometrists. The project received local ethics committee approval (Scarborough and North East Yorkshire Local Ethics Research Committee; Ref. No. PB/RH/02/288). The project adhered to the tenets of the Declaration of Helsinki for research on human subjects.

2.2.2 Eligibility Criteria

All individuals registered with a General Practitioner in Bridlington and 65 years and older on the 5th November 2002 were eligible for examination by the project. Subjects known to be moving in or out of the area during the study, those that were registered blind or partially sighted, bed bound individuals or those known to have significant dementia were excluded from the study. Subjects were invited by letter on a street-by-street basis in ascending numerical order of postcode. When contacted, each subject was invited to telephone the BEAP to make an appointment to be examined. Informed consent was obtained from all participants. The Project saw its first participant on 5/11/2002. By its completion in March 2006 over 3500 subjects had been examined. There were 3549 attenders, compared to 2770 non-attenders in the eligible population. Over 55% of the eligible population was examined. Analysis

of the BEAP database demonstrates Jarman index scores for participants in the project are similar to those of the non-attenders suggesting a good epidemiological sample. The non-attenders were slightly older (mean 75.3 years) compared with attenders (mean 73.4 years). The corresponding age ranges for non-attenders versus attenders were 65-102 and 65-99 years respectively. This difference is common among population-based studies. It is attributed to older subjects being reluctant to attend for assessment. If aged under 80 years, it was more likely an individual would attend than not. In this younger age group there was a mean difference in the Jarman score of 2.25. In BEAP subjects over 80 years, the Jarman score for attenders and non-attenders was similar. Apart from two individuals all subjects in the BEAP were Caucasian. The mean age of the cohort was 74 years.

The Jarman score for underpriviledged areas was developed as a measure of the general practitioners' (GP) workload. It has been used historically within health service planning, where it has helped in the allocation of resources, as a trigger for special payments to GP's along with use as a measure of urban deprivation [80]. In 1981 the Acheson committee reviewed primary care in London, collecting evidence on the social characteristics of the population, including factors such as how many people were living alone and its demand for primary care. It was the evidence gathered by this committee that formed the basis of the development of the Jarmin score [81, 82]. The Jarman score does have its limitations and critics. It has been documented to have a strong bias towards London, in terms of the proportion of the population classified as deprived [80].

The index has been criticized as failing to recognise the nature of social deprivation within Northern England, a bias that could result in unfair resource allocation for the benefit of the Thames regions [80]. The use of the Jarman index has also been extrapolated outside its original domain of intended use (as in this study), namely that of GP's workload to guide the allocation in healthcare resources [80].

Since the inception of this project other indices of deprivation have been developed and have replaced the Jarman index. When alternative deprivation indexes have been compared with the Jarman index, to determine the allocation of resources to doctors, there has been considerable variation [83]. The English Indices of Deprivation is one such tool (the fifth edition of a series of statistics) used to measure multiple forms of deprivation across a small spatial scale. If the BEAP was to be repeated, it is likely this deprivation scale would be used as a more modern alternative. A report on the technical details of the deprivation scale can be found online as published by the Department of Communities and Local Government (DCLG), who also provide information on how to use and interpret the Indices (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/465791/English_Indices_of_Deprivation_2015_-_Statistical_Release.pdf).

2.2.3 BEAP Examination Protocol

A trained nurse obtained a standardized medical history, including diabetes, stroke and hypertension, together with the subjects' drug history. Any history of amblyopia, ocular surgery or any other ocular disease was recorded. Distance and reading spectacle requirements were documented. Patients were asked specifically about a history of glaucoma, diabetic retinopathy or macular degeneration. Family history of glaucoma was determined as was the subjects driving status and social circumstances.

Log MAR visual acuities were obtained (with a Bailie Lovie number 4 chart) for each eye uncorrected, with glasses and with a pinhole. Each subject was examined by one of four optometrists trained specifically for the project. The optometrists provided their service on a daily rotational basis. A standardized slit lamp examination of the anterior chamber was performed followed by Goldman applanation tonometry. Subjects were subsequently dilated. Automated visual field analysis was then performed using the Henson Pro 5000 perimeter with software version 3.1.4 (Tinsley Instruments, Croydon, UK). A single stimulus, supra-threshold central 26-point test was employed. If a defect was detected this was automatically extended to a 68-point test. The Lens Opacities Classification 3 system (LOCS 3) was used to grade lens opacities[84]. The optic disc, macula and peripheral retina were examined using slit lamp

biomicroscopy and a 90D lens. Macular findings considered to be ARM/AMD were classified by the optometrists into the following categories. These were pigmentary changes, drusen, disciform scar and other. Optometrists were encouraged to use free text. Decisions on appropriate further management of each subject were then made. High resolution non-stereoscopic digital mydriatic fundus photographs were then taken for each eye with 30 degree fields centered on the fovea. A Topcon Fundus camera (model TRC NW6S) and a Nikon D1H (10 megapixel) digital SLR camera back was used. The images were stored on a hard drive. Heidelberg Retina Tomograph 2 disc images (HRT 2, Software Version 2.01, Heidelberg Engineering GmbH, Dossenheim, Germany) were also acquired.

All history and examination findings were recorded on a standard proforma. If the clinical findings were deemed to warrant further action then a referral proforma was completed to arrange review by the appropriate health care provider; either an optometrist, hospital eye service or general practitioner. The reason for referral was stated. When subjects were referred to the hospital eye service a proforma was completed by the reviewing clinician stating the diagnosis and further management.

2.2.4 Grading Training

In December 2013 I spent four weeks in Belfast at one of several reading centers, working in collaboration with the Central Angiographic Reading Facility (CARF). The CARF is a facility that co-ordinates the grading of a range of

ophthalmic images collected for various research studies, predominantly for AMD. The CARF then utilizes a network of reading centers located throughout the UK and electronically distributes the ophthalmic images for grading. There are three UK reading centers. These are located in London at Moorefield's Eye Hospital, in Liverpool at St Paul's Eye Unit in the Royal Liverpool University Hospital and in Belfast at the Queen's University. The CARF offers a range of services including the grading of color fundus photographs, FFA, indocyanine green angiograms and OCT images. The CARF also design grading protocols for the above imaging modalities as well as the training and certification of ophthalmic photographers and graders.

In the Belfast Reading Centre, I was trained in the grading of color fundus photographs to acceptable recognized standards, with a particular emphasis on the grading of AMD. Experienced and senior graders delivered training. The training involved the study of the official WARMGS manuals. These are an extensive collection of stereoscopic fundus photographic slides. They provide a stepwise training system from the normal fundus and variations of normal to drusen, pigmentary changes, GA and FV-PEDs and a range of other CNV. Slides are viewed with a stereoscopic viewer on a light box and are described with both an extensive text narrative and frequent illustrations. They emphasize the most important elements of grading along with common pitfalls. Following the completion of the entire WARMGS training manuals digital color fundus photographs were then graded alongside a senior grader.

2.3 Grading of retinal images

2.3.1 Background

Epidemiological studies estimating the prevalence of AMD rely on accurate methods of detecting the pathological changes of the disease. They also must use a standardized and consistent grading system. Although AMD had been described for over 100 years[1] no standardized agreement on its definition or a classification system had been developed. Early studies have been hampered by a lack of universally accepted diagnostic criteria and terminology [85-87]. Some authors have defined AMD using histopathological criteria, such as a continuous layer of basal laminar deposit under the macula [88, 89]. Others have previously defined it using a variety of different diagnostic tools such as dark adaptation, perimetry, contrast sensitivity, laser ophthalmoscopy, biomicroscopy, black-and-white or color fundus photography, FFA or indocyanine green angiography, among others[43]. There has also been inconsistency in the classification of the early stages of ARM. Some studies required either the presence of ARM features associated with a visual acuity of 6/9 or worse [90-93]; others did not [8, 10, 94]. The use of different diagnostic tools, definitions and severity scales limits comparison of prevalence rates across many early clinical and epidemiological studies.

With these problems in mind in 1995 the International ARM Epidemiological Study Group published a standardized procedure for retinal photography and grading[43]. The paper describes the results of a series of meetings of six groups involved in epidemiological studies of ARM. The aim was to allow an easier

comparison between studies in the future by adequately defining ARM and developing a grading system for color stereoscopic fundus transparencies. The grading system is based on the earlier WARMGS [27].

2.3.2 Photography and Film Processing

Historically, stereoscopic mydriatic color FP pairs centered on the disc and macula (respectively fields 1 and 2 of the modified Arlie House classification[95]) were mounted in clear plastic sheets. Grading relies heavily on the quality of stereoscopic transparencies, particularly for the detection of subtle drusen or pigmentary changes. Sometimes inclusion of field 3 (temporal to the fovea but including it in the nasal margin) would help detect subtle drusen and pigmentary changes or help decide whether visible changes were pathological or artefactual. Photographic film selection was very important. Kodachrome ASA 25 or 64 film or equivalent was historically used [41], because the film was deemed to have a high quality color and grain that was importantly stable over time [41]. Other films, such as Ektachrome 100 had previously been noted to be less stable over time, which has obvious implications for its use in long-term studies. Faster films with higher ASA grades appeared more 'grainy' [41] and films which contained too much red or brown could cause problems with grading RPE hypo and hyperpigmentation. It was recommended that the same film type (preferentially from the same batch and frozen at the start of the study) and film processor should ideally be used for epidemiological studies to minimize color shifts associated with processing or manufacture of films.

For epidemiological studies of ARM, the 30 or 35 degree photographic field is standard [41]. It provides an adequate magnification to determine most lesions associated with ARM. Any variation in photographic field size may result in difficulty comparing studies. Variation should be limited to between 25 and 40 degrees. Variation in image field also results in the need to adapt the grid template to the appropriate dimensions[96].

Historically slides were placed on a fluorescent viewing box containing a light with a Kelvin rating of approximately 6200 degrees. This light is bluer than the sun, which has a Kelvin rating of around 5400 degrees. This wavelength was chosen because of the observation that light of a lower Kelvin rating, with a more yellow hue, was less suitable for the identification of subtle drusen[27]. Graders historically examined slides with stereoscopic viewers, which mostly provide a 5 times magnification. Combined with the approximate 3 times magnification provided by the fundus camera, the result is a total magnification of 15 times.

With the development of digital imaging technologies and their subsequent incorporation into fundus cameras there has been a considerable shift away from the use of film transparencies for the study of ARM/AMD. The benefits from such a chance in practice are numerous. Firstly, digital imaging allows the photographer to judge the quality of the captured image instantly and to take further images in necessary without delay. Secondly processing and mounting 35mm slides on transparencies involves considerable skill and time. Slides use

storage space and images have to be retrieved manually, which is time consuming. Digital images can be stored on high capacity hard drives and are very easily transferred electronically to reading centers for analysis. Retrieval of digital data is almost instantaneous. The quality of a digital image does not degrade with time. Costs of digital cameras are now very reasonable and through the avoidance of film processing and slide mounting offer considerable cost savings. A graders posture when viewing a computer monitor is much more comfortable than when bent over viewing slides with a stereo-viewer and light box.

All of these advantages were considered at the beginning of the EUREYE study, which was an ARM prevalence study across eight European countries [23]. Up until this epidemiological project, no validation studies had been published comparing conventional film transparencies against digital imaging for ARM/AMD. Groups had previously investigated the use of digital imaging for the screening of diabetic retinopathy [97-102]. Most of these studies had suggested that digital imaging was equivalent or superior to film based image capture [97-100] or contact lens biomicroscopy [101]. One study, which used a non-mydriatic camera, concluded that digital fundus imaging was inferior to 35mm slide imaging [102]. Early signs of ARM such as pigmentary changes and small drusen may be more difficult to grade than features of diabetic retinopathy and therefore it was felt an instrument validation study was needed. Leeuwen et al (2003) were the first to report there were no important differences between both photographic techniques in the grading of ARM/AMD in an

epidemiological study setting. The between technique agreement ranged from good to very good and the between grader agreement was about the same for both techniques[103]. Following this publication, digital color fundus imaging has become the most commonly utilized imaging modality for epidemiological studies.

2.3.3 Grading

Throughout the BEAP, to maintain the quality of photography and grading of digital images, camera settings, software and monitor settings were not changed. Images were carefully stored with a backup on a separate hard drive. Digital non-stereoscopic mydriatic color FP with a 30-degree field were graded. Grading of such an image requires the use of a standard macular grid that allows the macular area to be outlined with internal subdivision that permits comparison of findings among different studies. Historically, epidemiological studies have used different grids with various sizes of concentric grid circles along with differing numbers and locations of radial lines on the grid[8, 40, 104]. Following the publication of the WARMGS [27] and the subsequent international classification and grading system by Bird et al[43] macular grids became standardized in their dimensions. The Early Treatment Diabetic Retinopathy Study (ETDRS) grid templates are based on the longstanding idea that the diameter of the average optic disc is 1500 micrometers, even though 1800-1900 micrometers may be a better estimate[105, 106]. The standard grid, formed by opaque lines is superimposed on a transparent background. Historically this

would then be superimposed over one member of the stereoscopic pair of field 2. It consists of 3 concentric circles centered on the fovea. The magnification produced by a 30 degree fundus camera is such that 4.7mm on the grid corresponds to approximately 1500 micrometers on the average fundus. The radius of the central circle corresponds to 500 micrometers in the fundus of an average eye. The radii of the middle and outer circles are 1500 and 3000 micrometers respectively. Four radial lines angled at 45 and 135 degrees divide the grid into nine subfields. There is the central subfield (within the inner circle) and then the middle and outer subfields, each of which is subdivided into superior, nasal, inferior and temporal subfields. Five open circles printed on clear plastic are used to estimate drusen size and area involved by drusen in the international grading system. The diameter of C_0 is equivalent to 63 micrometers. C_1 is 125 micrometers, and C_2, C_3 and C_4 are 175, 250 and 500 micrometers respectively.

2.3.4 Definitions for grading

ARM is a disorder of the macular area (within a circle with a diameter of 6000 micrometers, centered on the fovea), most often clinically apparent by the presence of drusen, hyper or hypopigmentation, without indication that they are secondary to another disorder. Examples of other pathologies to be excluded include retinal detachment, high myopia, chorioretinal infection, choroidal dystrophy, ocular trauma or inflammatory processes [43].

Drusen, are whitish-yellow deposits that are external to the RPE and the neurosensory retina. Their appearance varies considerably, merging at one end of their spectrum with the normal fundus background and at the other end regressing drusen merge with a diffuse degeneration of the RPE. Typical small drusen usually appear as individual flat round spots in the plane of the RPE. They can be pale yellowish-white lesions that contrast considerably with the surrounding RPE, or can appear only marginally paler than the background RPE and easily be overlooked[27]. Larger drusen are typically yellow-white in colour and often have visible thickness in stereoscopic pairs. Some have well defined margins with a nodular appearance, whereas others have indistinct margins and a softer, more liquid appearance[27]. Drusen may occur as individual spots or appear to merge with adjacent drusen and are termed confluent.

Drusen less than 63 microns are classified as hard drusen. They will be flat with well-defined margins and have no visible mass. Drusen over 125 microns are classified as soft. Soft distinct drusen have a uniform density with sharp edges. Soft indistinct drusen have decreasing density from their center outwards and have fuzzy margins. Drusen between 63 and 124 microns in diameter may be graded as either hard distinct or either of the soft categories.

One problem encountered with the grading of drusen is in defining the most-subtle appearance that can be classified as drusen. This problem was overcome in the WARMGS [27] by separating small drusen into two grades. Hard distinct drusen are small, less than 63 microns and are unequivocal. When the grader is 90 % certain that lesions are in fact drusen they should be graded as such. For

the BEAP, lesions that the grader feels are less than 50% likely to be drusen should not be graded. Hard indistinct drusen/questionable drusen (where the grader is between 50 and 90% certain that the lesion is in-fact a drusen) or questionable stippling should be graded as questionable and adjudication should take place. Stippling refers to the situation where the photograph demonstrates a background granular appearance where there is a diffuse scattering of tiny, ill-defined lighter spots than the surrounding RPE background. This may represent the physiological fundus background or tiny drusen. In the WARMGS, drusen where virtually all of their substance has disappeared are placed in a faded category. Faded drusen are distinguished from RPE degeneration by their separation from one another and their round or oval shape. If patches of highly confluent drusen were to become faded they would be graded as the appropriate type of RPE degeneration. In the BEAP faded drusen are not recorded as a separate category. Neither are calcified drusen.

RPD are defined as a network of broad-interlacing drusen forming a ribbon like pattern. There is no size component in terms of either minimum area involved throughout the fundus or size requirement for the component individual drusen within the reticular network. RPD may have enhanced visibility with blue light.

Areas of increased pigment or hyperpigmentation associated with drusen are classified as ARM. Areas of hypopigmentation of the RPE, most often more sharply demarcated than drusen, without any visible choroidal vessels associated with drusen are classified as ARM. The late stages of ARM shall be called AMD and this includes both 'dry' and 'wet' AMD[43].

'Dry' AMD or GA is defined as a sharply defined area of RPE loss that must be at least 175 micrometers in diameter and must be roughly round or oval in shape. It must have at least two of the following 3 features: have scalloped edges, have visible choroidal vessels that are more prominent than in the surrounding areas or have well defined margins in-keeping with the photo clarity.

'Wet' AMD, also termed 'neovascular' AMD (nAMD), 'disciform' AMD or 'exudative' AMD is characterized by any of the following lesions:

- Definite PED's, which may be associated with neurosensory retinal detachments, associated with other features of ARM. There is likely to be overlying atrophic change in the RPE and possibly associated haemorrhage.
- Subretinal or sub-RPE haemorrhages that are not associated with other vascular lesions. Intraretinal haemorrhages or breakthrough vitreous haemorrhage may also be graded as AMD if not attributable to other diseases.
- Hard exudates within the macula related to any of the above and not related to other retinal vascular disease.
- Intraretinal, sub-retinal or sub-RPE glial tissue. This will be evident by a white mass or retinal fibrosis within the macular grid that represents the scarring created by a chronic CNV within a FV-PED or a sub-retinal membrane. It is likely to be roughly round or oval in shape, but may be

interspersed with patches of atrophy or adjacent areas with more active features of AMD.

- A visible sub-retinal or sub-RPE membrane. This will be characterized by an area of grey/yellowish discoloration within the macular grid that is often ill defined. It may be associated with other features of AMD, but can be graded in isolation.
- Any of the above features, even if completely outside the macular grid, shall be recorded as PPCNV if directly adjacent and contiguous with the optic disc.
- When GA and nAMD coexist in the same eye, the said eye should be graded for nAMD.

If doubt exists and the grader is less than 50% sure the lesion could represent AMD, it should not be graded as such. If between 50-90% certain, the lesion is questionable and adjudication should take place. All lesions graded as GA or wet AMD need to be reviewed and scrutinized by Mr. Amoaku and the grade confirmed. He will also adjudicate any questionable lesions. If doubt exists as to whether a lesion resembles AMD or pathology such as diabetic retinopathy, pathological myopia, chorioretinitis or laser burns, then it should not be categorized as AMD.

Double masked grading of images would have been preferable. However, given financial limitations this was not possible. Quality assurance will be achieved through regular joint grading sessions with Mr. Amoaku and myself, with regular meetings to adjudicate questionable subjects. 1 in 10 randomly

selected images from the BEAP database will be sent to the CARF for secondary masked grading by a certified grader. Mr. Amoaku will adjudicate differences.

The signs of ARM/AMD shall be stratified using the Rotterdam grading system and recorded into one of five exclusive stages (0-4) [107, 108].

Grading of all fundus photographs takes place in a masked fashion. No knowledge of visual acuity or any other information such as subject demographics is known.

The grading results will be recorded for each eye. These AMD grades will then be correlated with visual acuities and other data from the BEAP. The prevalence of asymmetric AMD and asymptomatic AMD will then be measured. The ARM/AMD grade from color fundus photographic grading will be compared with that assigned by the examining/screening optometrist.

2.4 STROBE Statement

An international collaborative initiative of epidemiologists, methodologists, statisticians, journal editors and researches who were involved in the conduct and dissemination of observational studies developed STROBE with the aim of **ST**rengthening the **R**eporting of **O**bservational studies in **E**pidemiology (https://www.strobe-statement.org/index.php?id=strobe-home). The developers of STROBE point out that inadequate and incomplete reporting of research can significantly hamper the assessment of strengths and weaknesses of studies across medical literature. STROBE emphasise that readers of reports and journals must know what was planned, what was done, what was found and what the results mean. STROBE makes recommendations on the reporting

of studies; recommendations which are endorsed by several medical journals. STROBE publish a checklist of items that they recommend should be included in articles relating to observational research, such as cohort, case-control and cross-sectional studies. To be compliant with this process I have completed the STROBE checklist and included it as an appendix at the end of this thesis. It is completed with summary bullet point entries and in some instances makes direct reference to the thesis text in order to avoid repetition. All sections and contents are obviously covered in more detail throughout the thesis manuscript, but are briefly covered in the statement. The STROBE statement covers only the BEAP component of the thesis.

2.5 BEAP Study Bias

Like any cross-sectional study, the BEAP is susceptible to bias. This is briefly discussed below.

2.5.1 Selection Bias

The BEAP included a full list of eligible subjects (all those aged 65-years or older who were registered with the towns GP surgery). It was a complete cross sectional approach, with no sampling strategy. The results should therefore achieve the goal of being a more representative sample of the Bridligton elderly population, as compared to other approaches such as random or systematic sampling. Some individuals may have been left out of the study if indeed they were not registered with a GP, which would be unlikely given the age group. The most recent office of national statistics estimates that from 2015 the

population of England was 54.8 million. At the same time, the number of individuals registered with GP practices in England was 57.1 million-2.3 million more people (4%). It is not possible to explain this discrepancy for certain, but it likely represents a combination of over counting by GP's, under-counting of population estimates and nomenclature issues regarding who is a 'resident'. But, for whatever reason, it suggests that non-registration of subjects with GP's is likely to be a minority of the population. For the East Riding of Yorkshire region in 2015, GP registered subjects was 2% greater than the official population estimate (https://secondreading.uk/social-policy/population-estimates-gp-registers-why-the-difference/). It is a frequent finding, across almost all regions of the UK that more people appear to be registered with GP's than are actually resident. Qualitative research in the Royal Borough of Greenwich, estimates the population unregistered with a GP between 1.2% and 5.4% (http://www.greenwichccg.nhs.uk/Get-Involved/ppedocuments/Patient%20and%20public%20engagement%20documents/Queen%20Elizabeth%20Hospital%20A%20and%20E%20and%20UCC%20Research.pdf). No data is available from the Bridlington area.

There question as to whether Bridlington is a representative sample of the wider UK population is more difficult. Bridlington is predominantly a Caucasian population and the study offers no representation of the prevalence of AMD among different ethnicities and the results are less generalizable to other UK non-Caucasian populations. This is acknowledged as a limitation, but also strength of the study.

All eligible participants were contacted by letter. Therefore, selection bias in terms of individuals who only owned a phone was reduced.

2.5.2 Volunteer bias

In total 3549 individuals participated in the initial study examination, corresponding to 56% of the subjects within the eligible study population. This is similar to other studies with similar to other studies with an over 65 year population such as in the EUREYE, where the overall participation rate was 45.3% [23]. Therefore, the sample may be subject to volunteer bias, if the subjects who did not volunteer to attend were different from the attenders. A difference in characteristics between attenders and non-attenders is difficult to quantify but basic demographic information was available for all subjects within the sampling frame. Gender balance was similar for both attenders and non-attenders. There was a small but detectable difference in average jarman score (-3.92 vs -1.75) corresponding to slightly higher levels of deprivation among non-attenders but this was not judged to be clinically significant. At recruitment the non-attenders were slightly older (mean 75.3 years) when compared with the attenders (73.4 years) a difference that is common among population based studies and is attributed to older subjects being reluctant to attend for assessment. It has been reported that individuals who participate in studies are more educated, from a higher social class and are more sociable than those who do not participate [109]. We could have reduced volunteer bias by increasing the attempts to contact the patients or by using telephone contact for non-responders, but this was restricted by the ethics committee. The effect of our

low response rate and any bias relating to AMD status is uncertain. In particular it is unknown whether people already under the care of hospital eye clinics with AMD will be less likely to participate. A healthy participant effect such as this will reduce the prevalence estimate. We are not able to collect or quantify the visual status of non-respondents. Prevalence/incidence bias occurs when mild or asymptomatic cases are missed during studies. In the BEAP, this is not a concern as we used fundus photography as a means of assessing individuals for disease, so even asymptomatic individuals will be graded and recorded.

2.5.3 Information Bias

In the BEAP a trained research nurse questioned all patients on past medical history, including ocular diseases and relevant family history to attain risk factors and gather information. There is obviously the known tendency for respondents to provide what they believe as a socially acceptable answer rather than the truth. However, none of the questions explored topics/conditions associated with a great degree of social taboo, other than perhaps smoking. Recall bias may have been a more significant issue, relating to conditions such as hypertension or past smoking. Interviewer bias was minimized by standardizing the interviewers' interaction with the subjects.

2.5.4 Confounding

A pure prevalence survey does not examine causal relationships; hence confounding should not be a significant issue. Upon statistical analysis of associations, known confounders were addressed during data analysis, with regression analysis.

2.5.5 Outcome misclassification

During the study, the grading of eye disease was done with established objective diagnostic outcome measures by appropriately trained individuals. Quality assurance processes were put in place, with one in ten randomly selected right eye images undergoing secondary grading at the CARF, Belfast.

2.6 Sample Size

When designing epidemiological studies, it is important to choose a sample size that will detect effects being investigated, and to achieve the desired precision. It is essential that a sample is large enough to provide a precise and reliable answer to a study hypothesis, without being excessively large, resulting in inefficient wasting of study budget, time and resources[110-112]. Sample size calculations should be considered early in research designs. Methods of smaple size calculation differ between different study designs. One blanket formula cannot be used for all studies.

The sample size of population studied for the BEAP was not chosen by me, nor did I have any involvement in the conceptualisation and organisation of the BEAP. The population of Bridlington was chosen for several reasons. Firstly, the population was felt to have little immigration/emigration, resulting in a stable population which would result in low numbers of participants being lost from the project. Secondly, the area of Bridlington was serviced by one large GP surgery, whose GPs' were motivated and keen to partake in the project. The population was similar in size to other landmark studies previously published in foreign populations at the time. If I were to repeat a population based study

(like the BEAP today), assessing a qualitative variable like the prevalence of AMD I would use the formula as described by Charan et al (2013) in their paper which describes ample size calculations across a variety of study designs, as shown below [113].

2.6.1 Sample size formula for cross sectional studies

$$\text{Sample size} = \frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Here

$Z_{1-\alpha/2}$ = Is standard normal variate (at 5% type 1 error ($P<0.05$) it is 1.96 and at 1% type 1 error ($P<0.01$) it is 2.58). As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula.
p = Expected proportion in population based on previous studies or pilot studies.
d = Absolute error or precision – Has to be decided by researcher.

As a worked example, we can estimate the sample size required to measure thr prevalence of AMD in a UK population, with 95% confidence, and a margin of error of 5%, assuming an unlimited population size. Ffrom previous studies we know the prevalence of AMD is around 3.5% [23]. However, to take account of the more prevalent early changes, we know in the EUREYE Study, 47.59% of the population had no identifiable ARM [23]. We can calculate a sample size as below:

$$Sammple\ size = (1.96^2 \times 0.5241(1 - 0.5241)/0.05^2$$

=3.8416 × 0.5241 × 0.4759/0.0025

=383

Chapter 3: Prevalence of AMD in an elderly UK population-an overview.

3.1 Introduction

AMD is the leading cause of irreversible visual impairment in adult populations across developed countries [3], and remains a leading cause of global blindness [2]. With the increasing shift towards an ageing population, its prevalence is expected to increase significantly over the next decade. AMD represents the more advanced end of pathological changes that occur within the macular area with ageing. The earliest features of the ageing macula, referred to as ARM, consist of yellow sub-RPE deposits (drusen) some types of which have long been an established risk factor for the development of AMD since Gass first described the association in 1973 [114]. Focal alterations of the physiological pigmentation of the RPE also form part of the spectrum of ARM [107, 115]. The presence of drusen, with associated areas of focal hypopigmentation or hyperpigmentation of the RPE, have come to signify increased risk of progression to the more advanced stages of AMD, and the associated potential for visual loss [107, 115]. The two main phenotypes of AMD are GA, characterised by atrophy of the choriocapillaris, the overlying RPE and photoreceptors, and nAMD, which is signalled by the development of abnormal vascular proliferations CNV] within the subretinal or sub-RPE spaces. There are currently no proven treatments for dry AMD as yet. Despite treatments with anti-VEGF

therapies for nAMD, the number of individuals in the UK with sight loss secondary to AMD is expected to rise to 291 982 by 2020 [17].

Several population based studies from across the world have reported prevalence estimates for ARM and AMD [6-11, 14, 23, 116, 117]. These include mainly developed countries such as Australia [6, 7], the United States [8], and a significant number of European studies [9-11, 14, 23, 116, 117]. A few other studies have been reported from Asia and elsewhere [118-121]. Significant differences exist in the reported prevalence rates between these populations, reflecting either genuine differences and offering potential insight into genetic and environmental causes of AMD, or may simply represent the different study designs. Variations in age groups, photographic methods utilised (such as field size, stereoscopic/non-stereoscopic or mydriatic/non mydriatic photographs) along with variations in grading procedures and definitions may all potentially impact the reported prevalence. A recent meta-analysis of the prevalence of AMD across populations of European ancestry found a significant heterogeneity in prevalence rates between these studies [26]. Although there are several publications on the prevalence of AMD in the UK, there remains a paucity of data from population studies from this country. Some of the UK prevalence estimates are based on data from registration of visual impairment [19] which is associated with inherent shortcomings. Under-certification is a known issue [122], and while patients with bilateral visual impairment may be registered as such, patients with early ARM, asymptomatic, unilateral or eccentric AMD, where visual acuity (VA) is not reduced, may go unnoticed and unrecorded.

Furthermore, such registration is dependent on patient consent. Other estimates are based on pooled findings from studies carried out on various white populations of shared European ancestry in the UK and elsewhere [4, 17, 25]. Only a few studies on population based prevalence of AMD in the UK with large sample sizes over 1000 participants are available [20, 21, 24, 123], and much of the available data is old [12, 20]. Furthermore, the information provided on the early stages of ARM is sparse. The study by Evans et al [24] involved a large population (13, 900 people); however, the participants were restricted to individuals over 75 years of age and the study adopted community screening for reduced vision for their case detection, and only patients with pinhole VA below 6/18 were investigated for disease. A more recent publication by Ngai et al [22] included only male subjects aged 65-83 years and had 934 attenders. Like in other studies[123], the Speedwell eye study [22] formed part of the follow up of an ongoing longitudinal study commenced years previously. As such it may have the potential towards selection bias from a healthy survivor effect. Other studies have made use of non-standard grading definitions, again including reduced VA as a criteria, making comparisons to other populations difficult [21]. The EUREYE study included one UK centre in Northern Ireland, which contributed only 634 participants [23]. Akuffo et al recently reported for the first time the community prevalence of AMD in the Republic of Ireland. The Irish Longitudinal Study on Ageing (TILDA) age-specific prevalence for late AMD in the over 75 year age group of 2.2% seems lower than expected. The authors report the measured prevalence was similar to that in the National Health and Nutrition Examination

Survey [124]. This study shared one similar methodology that may contribute to the low prevalence; that of non-mydriatic 45° digital FP. The use of high resolution mydriatic stereo/non-stereoscopic digital imaging has been shown to be comparable at detecting AMD lesions when compared to film based stereoscopic photographs [125], but the use of 45° non-mydriatic photography has several shortcomings; it reduces colour contrast and results in an increased frequency of poor quality or ungradable images, especially in individuals with small pupils and media opacities (3). It has been shown to have a low sensitivity (70%) for detecting AMD when compared to 30° FP [126]. Despite this, in the TILDA study over 96% of photographs were deemed gradable which is higher than some published rates [127]. Dilation was not possible in the TILDA study as AMD grading was not the only aim, but this emphasizes that when feasible, the use of mydriatic FP is still the preferred option at detecting AMD in epidemiological studies. It makes comparison to other published prevalence rates easier to interpret which was one of the fundamental ideas behind standardising prevalence studies with the use of The International Classification System.

A recent systematic review, commissioned by the VISION 2020 UK Macular Interest Group to provide reliable estimates on AMD prevalence in populations of European ancestry [26], estimated the number of prevalent cases of AMD in the UK to be 539,000. Although to date this provides the most reliable estimate, a more robust population based measure will provide information on asymptomatic and asymmetric disease along with information on ARM,

allowing more adequate healthcare planning for the UK. This manuscript presents data on the prevalence of ARM and AMD in persons 65 years of age or older in a UK population.

3.2 Methods

3.2.1 Study Design

The BEAP is a single centre population based prevalence study, to investigate the utility of screening for eye disease in an elderly population using a combination of clinical examination and digital imaging technology. The primary ophthalmic diseases studied were AMD, cataract and glaucoma. Bridlington is a Yorkshire coastal town in the UK. This community was chosen because it includes a relatively stable elderly population with little migration. The study received approval from the local ethics committee (Scarborough and North East Yorkshire Local Ethics Research Committee; Ref No. PB/RH/02/288) and its methodology adhered to the tenants of the Declaration of Helsinki. A detailed description of the study design has been reported elsewhere [79]. Briefly, individuals aged over 65 years and registered with two general practice surgeries within the town of Bridlington were systematically invited to attend a screening visit. Subjects known to be registered blind or partially sighted, bed-bound or with significant dementia and those known to be moving in or out of the area during the study were excluded. Subjects were invited by letter on a street-by-street basis (in ascending numerical order of postcode) to telephone the project and make an appointment to be examined.

All participants were interviewed by either a trained research nurse or one of four specially trained optometrists using a structured questionnaire. Results were recorded on a proforma. The data recorded included demographic details, medical history including diabetes, hypertension, previous stroke and results of previous optometric assessment. A specific history of glaucoma, macular degeneration, diabetic retinopathy, previous ophthalmic operations and drug history was sought for each patient. All participants were asked if they were subjectively happy with their current level of vision.

LogMAR visual acuities were recorded for each eye corrected with both current glasses and pinhole (Baylie Lovie no.4 chart). Visual fields were performed with a Henson Pro 5000 analyser. A full biomicroscopic ophthalmic examination was then performed, including grading of lens status (LOCS 11), intraocular pressure, central corneal thickness and dilated fundus examination with a 90D lens. Macular findings considered to be AMD were graded by the optometrists into the following categories: pigmentary changes, drusen, disciform scar and other. Free text entry of additional findings was permitted. Results from individual patient proformas was anonymised and transcribed to an Excel (Microsoft, Redman, Wash) spreadsheet for subsequent analysis. A single researcher reviewed database entries for 10% of study proformas to determine the rate of transcription errors. The error rate for electronic database entry was found to be less than 0.1%. Non-stereoscopic mydriatic fundus photography was

performed with a Topcon fundus camera (model TRC NW6S) and a Nikon 10-megapixel camera. Each eye had a 30̊ colour FP taken centred on the macula.

3.2.2 Photographic grading

Photographs of both right and left eyes were graded using definitions and grids as described in the International Classification System for AMD [43] by a single ophthalmologist (CW) who was trained in image grading at the CARF, Belfast. Masked grading occurred with the graders being unaware of any demographic and medical history regarding the patients, including age and gender. In this system a circle with a diameter of 6000µm is centred on the fovea and features of ARM and AMD are recorded. The grid consists of 3 concentric circles with radii of 500, 1500 and 3000µm, with 4 radial lines angled at 45° and 135° that divide the grid into 9 subfields. Drusen were categorised on the basis of their size, homogeneity and outline[43]. Pigmentary irregularities were classified as either hyperpigmentation, hypopigmentation or both. GA was defined as a sharply demarcated area of RPE loss that was at least 175µm in diameter that must be roughly round or oval in shape, with at least 2 of the following features: scalloped edges, visible choroidal vessels that are more prominent than in the surrounding areas and well defined margins in-keeping with the clarity of the fundus photograph. nAMD was graded if there were any of the following features within the grid: definite RPE detachment, either haemorrhagic or serous and /or subretinal or sub-RPE haemorrhages that were not associated with any other vascular lesion and/or intraretinal, sub-retinal or

sub-RPE glial tissue and/or subretinal or sub-RPE neovascular membrane as characterised by a grey/yellowish discolouration. If any of these features occurred directly adjacent to and contiguous with the optic disc then a grade of PPCNV was made. When GA and nAMD coexist in the same eye, the said eye was graded for nAMD. All questionable lesions and all lesions that were graded as GA, nAMD or PPCNV were reviewed and scrutinised by a retinal specialist with expertise in image grading (WMA). Any differences in opinion were sent to CARF for grading. Frequent sessions of simultaneous grading were performed to maintain reproducibility. If doubt existed as to whether a lesion resembles AMD or other pathology such as diabetic retinopathy, pathological myopia, chorioretinitis or laser burns, then it was not graded as AMD. All images in this study graded as GA were specifically reviewed to ensure that none had a recorded history of diabetic retinopathy or previous macular laser. The signs of ARM/AMD were stratified using the Rotterdam grading system (
Table 1) and recorded as one of five exclusive stages (0-4) to facilitate statistical analysis[107, 108]. 1 in 10 randomly selected right eye images from the BEAP database were sent to the CARF in Belfast for secondary masked grading by a certified grader. Any differences were adjudicated by WMA. Each eye was graded separately, and the final grade assigned to each participant was that of the worse eye.

Statistical analysis was performed using Stata 12.0 (StataCorp, College Station, TX) and SPSS v.22 (IBM Corp. Armonk, NY)

Of note is the use of a modified Rotterdam Grade, which was assigned to each eye as the final grade. This term was chosen for a few reasons as briefly discussed and illustrated below in Table 1 below. Heterogeneity secondary to methodological differences among studies can occur, and can relate to differences in classification of disease phenotypes and grading protocols. This becomes an issue, particularly if pooling data for the performance of meta-analysis. It is therefore important that I highlight the precise differences here in detail. Firstly, the Rotterdam study (RS) used stereoscopic photographs (35°) of both ETDRS fields 1 and 2 [10]. In our study we utilised non-stereoscopic FP of field 2 only. Another difference was that unlike in the RS we utilised digital FP, as opposed to colour transparencies and a viewing box. A grading difference was the sub-classification of grade 4 into 4a/b/c in the BEAP. It was felt important to record the differences and also record PPCNV as distinct from nAMD affecting the macula area, since no literature existed on the prevalence of PPCNV. Another significant grading difference is that in the RS, 'no ARM' was assigned to any eye that had no ARM features or only drusen ≤63μm. In the BEAP, this group was subdivided into those with no ARM features whatsoever (0a) and those with <10 hard drusen ≤63μm (0b). An extra category (≥ 10 hard drusen) was added within the 1a grouping for the BEAP. In the International Classification System small drusen are not considered an ARM feature [43]. Since then however, data from the RS has indicated that more than 10 small drusen is predictive of ARM progression, independent of other features [108]. This finding is consistent with findings from the Waterman Study [128] and the BDES [8], which

both report the presence of many small drusen increase the risk of large and soft indistinct drusen, but not AMD.

For the BEAP, size categories were added within groups to aid subclassification, given the absence of stereoscopic FP, which can make the identification of distinguishing features of soft and indistinct drusen more difficult.

3.3 Results

Study recruitment took place between 5/11/2002 and 29/03/2006. In total 3549 individuals participated in the initial study examination, corresponding to 56% of the subjects within the eligible study population. Basic demographic information was available for all subjects within the sampling frame. Gender balance was similar for both attenders and non-attenders. There was a small but detectable difference in average jarman score (-3.92 vs -1.75) corresponding to slightly higher levels of deprivation among non-attenders but this was not judged to be clinically significant. At recruitment the non-attenders were slightly older (mean 75.3 years) when compared with the attenders (73.4 years) a difference that is common among population based studies and is attributed to older subjects being reluctant to attend for assessment.

A total of 3475 attenders had gradable photographs in at least one eye. 300 individuals had ungradable images in at least one eye, including 74 individuals who had ungradable images in both eyes. The mean age of those with gradable

photographs was 75 years (SD 5.9, range 65-100, 95% ci 74.8 - 75.2). Individuals with ungradable photographs tended to be older, with a mean age of 77.7 years (SD 6.0, range 66-95, 95% ci 77-78, $p<0.001$ mann whitney u test). There was a slightly increased female preponderance (58:42) in the ungraded group but this difference did not reach conventional levels of statistical significance ($p=0.46$, chi2).

Interobserver variability was assessed using Kappa. Using the cut-offs proposed by Landis and Koch [129], it was found that there was substantial agreement between CARF and CW, with 76% agreement (kappa=0.69, SE 0.03, $p<0.001$) and excellent agreement between CW and WMA, with 86% agreement (kappa=0.82, SE 0.04, $p<0.001$). There was good agreement between graders across all stages of AMD. The interpretation for the higher grades was limited by the low number of study participants. The combined kappa for all 3 graders (CARF, CW and WMA) for all categories was 0.71.

This is not dissimilar to previously published reports. Using a revised version of the grading system established by the International ARM Epidemiological Study Group for identifying abnormalities of ARM/AMD, Scholl et al published similar measures of Interobserver variability between 3 retinal specialists using stereoscopic FP [130]. Inter-observer agreement generally increased with more advanced stages of disease being fair to substantial for small hard drusen (70–89%; $\kappa=0.26–0.63$) and intermediate soft drusen (76–94%; $\kappa=0.27–0.69$). Agreement ranged between 87% and 100%, between 50% and 92%, and between 78% and 100% for larger drusen, the presence of hyperpigmentation,

and the presence of hypopigmentation, respectively. Agreement was moderate to almost perfect for the presence of GA (88–98%; κ=0.60–0.95) and substantial to almost perfect for the presence of CNV (84–100%; κ=0.62–1.00) [130]. In the National Health and Nutrition Examination Survey the degree of agreement between graders ranged from 66.0% to 73.0% for drusen characteristics and 88.0% or greater for other AMD characteristics, with kappa being generally moderate to substantial (0.48-1.00) [124]. In a Norweigian prevalence study (the Tromso Study) the Interobserver exact agreement was 63% (Kappa 0.48) [131]. In TILDA the intragrader reliability showed moderate agreement for all categories. Kappa scores varied from 0.51 to 0.61 [117]. The higher agreement between CW/WMA (86%) likely represents potential bias secondary to regular sessions of simultaneous grading. It is recognised that WMA is not a trained grader but a highly experienced retinal specialist. This may also be a source of bias. Prevalence rates were very similar between the right and left eyes as illustrated in Table 3 and did not differ significantly between the genders. Almost 40% of subjects had no or minimal (<10 small hard drusen <65µm in size) signs of ARM/AMD in their worse eye (grade 0). Individual eyes were more likely to be grade 0, with approximately 50% of eyes having no significant ARM. When graded for the worse eye however, a higher prevalence of grade 1 (41.4%) occurred as shown in Table 2. Prevalence rates for the worse eye were: 12.8% for grade 2 and 2.8% for grade 3; GA or nAMD (grade 4 AMD) was diagnosed in 149 persons, giving a prevalence of 4.3%. For the worse eye, GA (grade 4a) was more prevalent (2.5%) than nAMD (grade 4b) (1.8%) as shown in Table 2. These

differences in prevalence rates were significantly greater when analysed on an individual eye basis, with GA being of almost twice the prevalence for both the right and left eyes (Table 2) indicating significant disease asymmetry (see below). As shown in Table 3, although 60% of prevalent cases of nAMD were in females, when adjusted, the percentage prevalence within each gender was 2.0% and 1.7% for females and males respectively. PPCNV (4c) was an infrequent finding, with prevalence for the worse eye of 0.3%.

As shown in Table 4, the prevalence of the earlier stages of ARM decreases with age. In subjects aged 65 to 69 years, 44.5% had grade 0 AMD in their worse eye, but in the over 90 year age group only 15.2% of subjects had no or minimal morphological changes evident. There is a statistically significant increase in AMD prevalence with increasing age from 65 years upwards as shown in Table 4. As expected, the prevalence of grade 4 AMD increased from 2.2% in the 65-69 year age group, to 15.9% for individuals aged 85-90 years. This reached its maximum prevalence of 21.2% in the over 90 years' age group. As the stage of ARM/AMD increases, the mean age of subjects with that stage of disease increases as shown in Table 5. This is true for the right eye, left eye and the worse eye grades.

There was a positive correlation of ARM/AMD grades between the two eyes of individual participants as indicated by a Pearson's correlation of 0.5138. There was however significant disease asymmetry. Of the 85 persons with GA in at least one eye, only 32 subjects (37.7%) had bilateral GA. There were a total of 64 persons having nAMD in at least one eye. Bilateral disease was present in 14

subjects (21.9), indicating that nAMD was more likely to be a unilateral finding when compared to GA. Fifteen (15) people (23.4%) had GA in one eye and nAMD in the other. One individual had GA in one eye and a PPCNV in the other, while another had nAMD in one eye and a PPCNV contalaterally. Community prevalence for bilateral GA and nAMD was 0.9% and 0.4% respectively. Bilateral AMD (either 4a, 4b or 4c) occurred in 63 people overall (1.8%).

The proportion of the population with self-perceived dissatisfaction with vision is shown in Table 6. As the grade of ARM/AMD increased so did the self-perceived dissatisfaction with vision. It appears that a significant number of participants with the more advanced grades of ARM/AMD still considered their vision to be satisfactory, with 61.0% and 40.6% of subjects with known GA and nAMD respectively still feeling their vision was satisfactory when asked. It is apparent from Table 6 that in the early stages of ARM, the majority of subjects were happy with their vision. As the grade of ARM/AMD for the worse eye increased, the percentage of subjects happy with their vision decreased from 77.9% (Grade 0) to 40.6% for grade 4b; the latter grade being the only stage at which the majority were dissatisfied with their vision. When considering the better eye there is a significant increase in subject dissatisfaction with vision, particularly for individuals with bilateral nAMD. Table 7 demonstrates that individuals with AMD in their better eye (grade 4a or 4b) are over 4 times more likely to be report feeling dissatisfied with their vision when compared to individuals with early ARM (grade 0).

As shown in Table 8, spectacle corrected visual acuity (SCVA) was well maintained through the early stages of ARM, with a visual acuity of Log MAR 0.3 or better for most eyes within these early stages. Even in eyes with GA, (grade 4a) vision was maintained to a level of Log MAR 0.3 or better for 36.4% and 41.3% of right and left eyes respectively. There was significant variation in vision, ranging from excellent (LogMAR 0.0) to counting fingers (CF), depending on the exact location of the degeneration. As expected, eyes with nAMD (Grade 4b) were the most likely to suffer significant visual impairment, with 57.9% and 55.0% of right and left eyes respectively having a SCVA of LogMAR 1.0 or worse. Only a small minority of subjects with nAMD (10% and 5%) maintained a good SCVA in the right and left eyes respectively. There was no association of AMD grade with gender ($p=0.55$), the presence of diagnosed hypertension ($p=0.513$), or diabetes mellitus ($p=0.882$). A history of a previous stroke did show a trend but for the left eye only ($p=0.055$). As the right eye showed no trend ($p=0.318$) the significance of this finding is uncertain and it may reflect chance.

The relationship between AMD and cataract was explored using linear and logistic regression models using the grade of nuclear cataract and the presence of significant cataract as the respective dependent variables. Significant cataract was defined as LOCS 3 nuclear sclerosis ≥4 or cortical cataract ≥3 or posterior subcapsular cataract ≥2 or evidence of previous surgery. A weak association between AMD and cataract was found but disappeared when age was included as a covariate.

Information from the local social services department (East Ridings) have confirmed that there were 138 persons aged 65 years or more registered as severely visually impaired or visually impaired at the commencement of this project (2003) in the post code area of this study. However, a more detailed breakdown of this information to allow the identification of people with AMD excluded from the study is unfortunately unavailable. Our search was able to identify only 4 cases visual impairment due to AMD. Published literature suggests that approximately 50% of registrations for visual impairment or severely visually impaired are attributable to AMD [19, 132].

3.4 Discussion

This is the largest population-based screening study of AMD in the UK to date in an over 65-year population, and includes a significant number of participants that had gradable photographs in the over 80 year age group. This represents a scarce new finding among current published UK population studies, where older subgroups tend to be small. Furthermore, the prevalence measurements in this study are based on results from a large number of gradable fundus photographs. Grading and adjudication was performed by appropriately trained ophthalmologists, predominantly by one, reducing inter-grader variability. Quality assurance through secondary grading was also carried out by an established reading centre. It makes use of digital fundus photography and grading methods that are well recognised and used in previous epidemiological population based studies, including the EUREYE and Rotterdam Studies[23, 133]. All fundus grading was performed in a masked fashion, without knowledge of VA or

any other patient demographics. Despite the elderly cohort, photographs were generally of an excellent quality, reflecting the use of experienced photographers and the utilisation of mydriasis. The use of mydriatic fundus photographs in this study obviated the disadvantages of non-mydriatic photography, utilised by some others [117, 123], which could hamper the grading of particularly early ARM.

The measured prevalence of advanced AMD (grade 4) in the over 65 year population was 4.3%, which is higher than that reported in several other comparable studies such the EUREYE (3.77% in the Belfast arm) and in the Speedwell eye study (0.5%) but very similar to that of 4.8% derived from the Owen analysis of 2012 [25]. This is despite the exclusion of participants who were registered as visually impaired, some from AMD. The prevalence rates of AMD in this BEAP study are generally higher than previously reported in similar age subgroupings in other studies, as shown in Table 9. In the Rotterdam study, in the over 85 year population the prevalence of late AMD was 11.1% compared to 15.3% and 21.2% for the 85-90 and over 90 years age groups in the present study. These prevalence figures from the present study are also considerably higher than the Owen et al estimates for the corresponding age groups. To the best of our knowledge, this study provides for the first time, the prevalence figures for grade 4c AMD in the UK.

This study provides data on the prevalence of early stages of AMD and the percentage of patients with asymmetric AMD, i.e. advanced disease in one eye and near normal vision in the other eye. Disease asymmetry occurred in as

many as 60% of participants. This indicates that there's a significant number of the population who may have advanced AMD in the one eye whilst the other eye was functioning normally. These individuals will not be captured by the current methods of visual impairment data collection in the UK. This information should help inform adequate health care planning and provision for patients with macular disease. This study confirms that persons with the earlier and intermediate stages of AMD are asymptomatic. As such the presence of such early grades of ARM/AMD can only be detected by regular annual checks by the primary eye care service.

It is known that phenotypes of AMD can differ between different communities and populations. A common finding among previous prevalence studies from populations of or derived from European ancestry is that nAMD is more common than and, sometimes almost twice as common as atrophic AMD/GA [6, 8, 10, 23]. The TILDA Study showed that GA and nAMD had similar prevalence in the Irish population of >50 years, although these prevalence's were significantly lower than in our study, possibly reflecting the utilisation of non-mydriatic photography, making the acquisition of clear images difficult with the presence of media opacities [117]. Our findings indicate that the prevalence of GA is not less common than nAMD. In this BEAP study there is a reversal of this pattern, with GA being 1.7 and 1.6 times more prevalent in the right and left eyes respectively. This difference remains for the worse eye, with GA still being 1.3 times more common. This finding is similar to that reported elsewhere, typically in Iceland [14, 134]. Jonassen et al (2003) in the Reykjavik Eye Study

(Iceland) reported that for individuals over 70 years of age, atrophic AMD and nAMD occurred in 9.2% and 2.4% respectively [14]. In the Greenland Inuit Eye Study Andersen et al (2008) this finding was not repeated with GA occurring in one or both eyes in 2.3%, nAMD in one or both eyes in 6.1%, and GA in one eye and nAMD in the other eye in 1.1% of the 695 participants aged 60 years or over. The nature and significance of these differences is uncertain but could reflect genetic factors which have been reported to play a role in the prevalence of AMD [135-137]. In the Reykjavik Eye Study subjects with GA were found to be a greater likelihood of being related than would be expected from a random sample, having more recent common ancestors in their family pedigrees. We do not have such family demographic data but it may be possible that a similar factor, such as limited migration and shared common ancestry are responsible for the findings in our study. Other explanations could be the mis-classification of chronic PED's associated with areas of GA (potentially secondary to the utilisation of non-stereoscopic photography) or previous laser photocoagulation in the macular area. Our re-evaluation of images, however, excluded any possibility of mis-classification or confounding with previous laser treatment. We retrospectively reviewed the BEAP data for all patients with GA and none had a recorded history of diabetic retinopathy or previous macular laser.

The self-reported satisfaction with participant's vision is interesting. It shows that a large proportion of individuals (61.0%) with GA in their worse eye still remain satisfied with their vision. When looking at the worse eye, nAMD is the only stage at which the majority of participants are dissatisfied (59.4%). When

considering the better eye there is a significant increase in subject dissatisfaction with vision, particularly for individuals with bilateral nAMD with almost 90% of subjects being dissatisfied. We used a logistic regression model to identify factors associated with dissatisfaction with vision. We found that age, cataract and AMD were all associated. Those with AMD were five times more likely to be dissatisfied with vision. When corrected for the effects of age and cataract, those with AMD were four times more dissatisfied with vision than those without.

Our results are likely to be highly representative of the population studied, with 56% of the eligible population being examined, and Jarman scores (indicating similar measures of deprivation) that were similar between attenders and non-attenders. The attendance rates are largely comparable to other studies, [7, 10, 94, 117] which is particularly good considering many of these used a lower minimum age for inclusion. It is well known in epidemiological research that attendance rates decrease with increasing participant age. This is reflected to some degree in our attenders being on average slightly younger. Allowing home examination would have perhaps increased our response rate. However, this would have reduced the degree of standardisation of procedures and grading through exclusion of photography, and was ultimately not possible because of ethical constraints.

One limitation of the current study is that the photographs were non-stereoscopic, which could have resulted in an underestimation of the prevalence of nAMD by missing subtle PED's. However chronic PED's associated

with significant activity from a CNV would likely be associated with other signs such as retinal haemorrhages or gliosis which would have allowed their identification. As this study excluded persons who were registered as severely visually impaired and visually impaired, a significant number of patients with advanced AMD in both eyes may have been excluded. This implies that our reported figures for the prevalence of advanced AMD (grade 4), which are higher than those previously reported, may actually be an underestimation of the true prevalence. In the BEAP study several participants had significantly reduced vision bilaterally but were not registered as visually impaired. This finding does suggest that at the time of the project visual impairment registration data may have been incomplete in the Bridlington area. The other limitation of this study is that the population was purely Caucasian, and therefore, does not provide any information on racial differences in AMD in the UK. However, this is also strength, as a similar sized study of a mixed population would have wider confidence limits for each finding in each racial group.

In conclusion, this study provides contemporary prevalence rates of different stages of AMD in a UK population, and indicates that the prevalence of advanced AMD is more common than previously thought. It has also, for the first time, provided data on the occurrence of AMD asymmetry in the population. Further studies are required in other UK communities to determine differences in prevalence amongst the different ethnicities, as well as determine incidence rates.

Table 1: Modified Rotterdam AMD Grading Scale and Original Rotterdam grade

	Modified Rotterdam Study Grade (BEAP)	Rotterdam Study Grade
Grade	Description	
0a	Normal-no signs of AMD at all	Stage: 'No ARM' No ARM features or only drusen ≤63µm
0b	<10 hard drusen <63µm in size	No Such grade
1a	≥10 hard drusen or any soft distinct drusen ≥63µm	Soft, distinct drusen
1b	Pigmentary abnormalities only, or with hard drusen 63µm in size, no soft drusen	Pigmentary irregularities
2a	Soft distinct drusen ≥125µm in size or reticular drusen only	Soft, indistinct, or reticular drusen
2b	Soft distinct drusen ≥63µm in size with pigmentary abnormalities	Soft, distinct drusen with pigmentary irregularities
3	Soft indistinct drusen ≥125µm with pigmentary abnormalities	Soft, indistinct, or reticular drusen with pigmentary irregularities
4a	GA	4: Grouped Atrophic or neovascular macular degeneration (AMD)
4b	nAMD	
4c	PPCNV	
7	Other macular disease	
8	No image available	
9	Ungradable image	

Table 2: Prevalence of worse BEAP AMD grade for right eye, left eye and worse eye.

Data is number (percentage) [95% Confidence Interval (CI)].

Grade	Right Eye	Left Eye	Worse Eye	Best eye
0	1689 (50.57) [48.87-52.26]	1733 (51.21) [49.53-52.89]	1337 (38.47) [36.87-40.10]	2195 (63.17)
1	1136 (34.01) [32.42-35.64]	1115 (32.95) [31.39-34.55]	1440 (41.44) [39.81-43.09]	896 (25.78) 24.36-27.27]
2	340 (10.18) [9.20-11.25]	339 (10.02) [9.05-11.08]	443 (12.75) [11.68-13.90]	255 (7.34) [6.52-8.25]
3	66 (1.98) [1.55-2.51]	90 (2.66) [2.17-3.26]	97 (2.79) [2.29-3.40]	66 (1.90) [1.49-2.41]
4a	66 (1.98) [1.55-2.51]	63 (1.86) [1.45-2.38]	85 (2.45) [1.98-3.02]	48 (1.38) [1.04-1.83]
4b	38 (1.14) [0.83-1.56]	40 (1.18) [0.87-1.61]	64 (1.84) [1.44-2.35]	15 (0.43) 0.26-0.72]
4c	5 (0.15) [0.05-0.36]	4 (0.12) [0.03-0.32]	9 (0.26) 0.13-0.50]	0 (0) [0.00-0.13]
Total	3340 (100)	3384 (100)	3475 (100)	3475 (100)

Table 3: Sex distribution of worse eye Rotterdam AMD grade.

Data is number (percentage) with gender specific prevalence ***in bold italics***. [95% CI]

Grade	Females	Males
0	733 (54.8) ***37.8.%*** **[35.7-40.0]**	604 (45.2) ***39.32%*** **[36.9-41.8]**
1	794 (55.1) ***40.95%*** **[38.8-43.1]**	646 (44.9) ***42.06%*** **[39.6-44.5]**
2	259 (58.5) ***13.36%*** **[11.9-15.0]**	184 (41.5) ***11.98%*** **[10.5-13.7]**
3	61 (62.9) ***3.15%*** **[2.5-4.0]**	36 (37.1) ***2.34%*** **[1.7-3.2]**
4a	48 (56.5) ***2.48%*** **[1.9-3.3]**	37 (43.5) ***2.41%*** **[1.7-3.3]**
4b	38 (59.4) ***1.96%*** **[1.4-2.7]**	26 (40.6) ***1.69%*** **[1.2-2.5]**
4c	6 (66.7) ***0.31%*** **[0.12-0.69]**	3 (33.3) ***0.2%*** **[0.04-0.6]**
Total	1939 (55.8)	1536 (44.2)

Table 4: Age distribution by worse eye Rotterdam AMD score.

Data is numbers (percentage within age categories) [95% CI]

	Age, years						
AMD score	65-69	70-74	75-79	80-84	85-90	≥90	Total
0	378 (44.52) [41.21-47.88]	450 (42.10) [30.17-45.08]	285 (35.27) [32.05-38.63]	166 (31.14) [27.36-35.20]	53 (28.96) [22.86-35.93]	5 (15.15) [6.17-31.4]	1337 (38.47) [36.87-40.10]
1	371 (43.70) [40.40-47.06]	478 (44.71) [41.76-47.71]	344 (42.57) [39.21-46.01]	189 (35.46) [31.51-39.61]	50 (27.32) [21.37-34.21]	8 (24.24) [12.60-41.25]	1440 (41.44) [39.81-43.09]
2	70 (8.24) [6.57-10.30]	105 (9.82) [8.10-11.76]	111 (13.74) [11.53-16.29]	109 (20.45) [17.24-24.09]	39 (21.31) [15.97-27.83]	9 (27.27) [14.90-44.39]	443 (12.75) [11.68-13.90]
3	11 (1.30) [0.70-2.33]	15 (1.40) [0.83-2.32]	25 (3.09) [2.08-4.55]	30 (5.63) [3.95-7.95]	12 (6.56) [3.69-11.22]	4 (12.12) [4.21-27.93]	97 (2.79) [2.29-3.40]
4a	9 (1.06) [0.53-2.04]	16 (1.50) [0.0-2.44]	21 (2.60) [1.68-3.96]	18 (3.38) [2.11-5.31]	18 (9.84) 6.24-15.09]	3 (9.09) [2.37-24.34]	85 (2.45) [1.98-3.02]
4b	8 (0.94) [0.44-1.88]	4 (0.37) 0.11-1.00]	19 (2.35) [1.49-3.67]	19 (3.56) [2.26-5.53]	10 (5.46) 2.88-9.88]	4 (12.12) [4.21-27.93]	64 (1.84) [1.44-2.34]
4c	2 (0.24) [0.01-0.91]	1 (0.09) [0.01-0.58]	3 (0.37) [0.07-1.14]	2 (0.38) [0.01-1.45]	1 (0.55) [0.01-3.34]	0 (0.00) [0.00-12.39]	9 (0.26) [0.13-0.50]
Total	849 (100)	1069 (100)	808 (100)	533 (100)	183 (100)	33 (100)	3475 (100)
P-value	<0.001						

Table 5: Mean age distribution of Rotterdam grades.

	Right eye			Left eye			Worse eye		
AMD grade	N	Mean (SE)	95% CI	N	Mean (SE)	95% CI	N	Mean (SE)	95% CI
0	1689	73.9 (0.13)	73.6-74.2	1733	74.2 (0.13)	73.9-74.4	1337	74.1 (0.15)	73.8-74.4
1	1136	74.7 (0.16)	74.4-75.0	1115	74.5 (0.17)	74.2-74.8	1440	74.4 (0.14)	74.1-74.7
2	340	77.9 (0.35)	77.3-78.6	339	77.6 (0.34)	77.0-78.3	443	77.2 (0.30)	76.7-77.8
3	66	79.2 (0.84)	77.5-80.9	90	79.4 (0.74)	77.9-80.8	97	78.9 (0.69)	77.6-80.3
4a	66	79.9 (0.84)	78.2-81.6	63	79.8 (0.87)	78.1-81.6	85	79.5 (0.75)	78.0-81.0
4b	38	80.6 (0.97)	78.6-82.6	40	79.4 (1.06)	77.3-81.6	64	79.8 (0.81)	78.2-81.4
4c	5	76.2 (2.90)	68.1-84.2	4	76.1 (3.88)	63.7-88.6	9	76.1 (2.20)	71.1-81.2
Total	3340	74.9 (0.10)	74.7-75.1	3384	74.9 (0.10)	74.7-75.1	3475	75.0 (0.10)	74.8-75.2
P-value	<0.001			<0.001			<0.001		
7	70	76.2 (0.59)	75.0-77.3	46	77.2 (0.83)	75.5-78.9			
8	40	77.5 (0.97)	75.5-79.5	36	77.8 (1.08)	75.6-80.0			
9	87	79.3 (0.66)	77.9-80.6	71	78.4 (0.79)	76.8-80.0			

Table 6: Subject self-perception of vision as being satisfactory, with corresponding AMD grades.

	Vision perceived as satisfactory by study subject worse eye vision		Percentages not satisfied with vision stratified by better eye grade
AMD score	No Number (%)	Yes Number (%)	
0	291 (22.06)	1028 (77.94)	458 (21.3)
1	286 (20.30)	1123 (79.70)	200 (22.6)
2	116 (26.36)	324 (73.64)	76 (30.0)
3	31 (33.33)	62 (66.67)	27 (43.5)
4a	32 (39.02)	50 (60.98)	23 (48.9)
4b	38 (59.38)	26 (40.62)	13 (86.7)
4c	3 (33.33)	6 (66.67)	0 (0)
Total	797 (23.33)	2619 (76.67)	
P-value Pearson Chi-Square	<0.001		

Table 7: Odds ratios for dissatisfaction with vision when compared to subjects with grade 0a/0b

AMD grade	Crude unadjusted odds ratio			Logistic regression model					
	Better eye			Better eye adjusted for age and significant cataract			Worse eye adjusted for age and significant cataract		
	Odds ratio	P>chi^2	95% CI	Odds ratio	P>z	95% CI	Odds ratio	P>z	95% CI
0	1.00			1.00			1.00		
1	1.08	0.40	0.90-1.31	1.06	0.58	0.87-1.28	0.90	0.28	0.75-1.09
2	1.59	0.00	1.19-2.12	1.38	0.04	1.02-1.86	1.17	0.23	0.90-1.51
3	2.86	0.00	1.71-4.78	2.49	0.00	1.48-4.22	1.56	0.06	0.98-2.46
4	5.13	0.00	3.05-8.63	4.35	0.00	2.57-7.35	2.73	0.00	1.92-3.88
Visually significant cataract[ǂ]				1.31	0.00	1.11-1.55	1.30	0.00	1.10-1.54
Age (per year increase)				1.02	0.00	1.01-1.04	1.03	0.00	1.01-1.04

ǂ visually significant cataract was defined as LOCS 3 nuclear sclerosis ≥4 or cortical cataract ≥3 or posterior subcapsular cataract ≥2 or evidence of previous surgery.

Figure 1: Chart depicting prevalence of AMD by age-group.

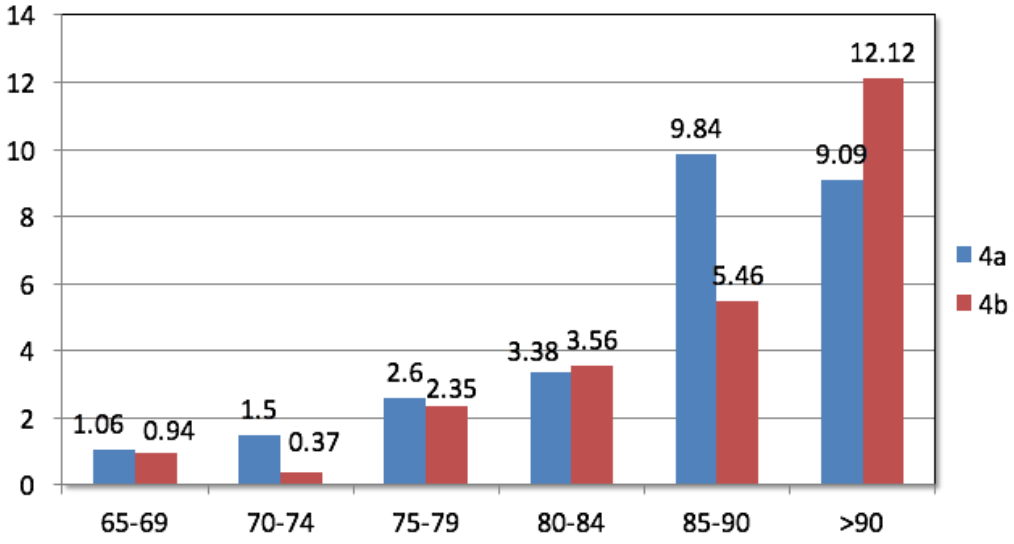


Table 8: Summary of spectacle corrected visual acuity (SCVA) for right and left eyes across AMD grades, subcategorised for level of visual impairment.

Data are numbers (row percentage).

Rotterdam Grade	Right eye SCVA				Left eye				
	Good vision	Low vision	Poor vision	Total	Good vision	Low vision	Poor vision	Total	
0	1257 (74.4)	416 (24.6)	16 (0.95)	1689 (100)	1267 (73.1)	439 (25.3)	27 (1.6)	1733 (100)	
1	852 (75)	280 (24.7)	4 (0.35)	1136 (100)	833 (74.7)	268 (24.0)	14 (1.3)	1115 (100)	
2	213 (62.7)	123 (36.2)	4 (1.2)	340 (100)	222 (65.5)	113 (33.3)	4 (1.2)	339 (100)	
3	28 (42.4)	38 (57.6)	0 (0.0)	66 (100)	48 (53.3)	40 (44.4)	2 (2.2)	90 (100)	
4a	24 (36.4)	30 (45.5)	12 (18.2)	66 (100)	26 (41.3)	27 (42.9)	10 (15.9)	63 (100)	
4b	4 (10.5)	12 (31.6)	22 (57.9)	38 (100)	2 (5.0)	16 (40.0)	22 (55.0)	40 (100)	
4c	3 (60.0)	2 (40.0)	0 (0.0)	5 (100)	1 (25.0)	3 (75.0)	0 (0.0)	4 (100)	
Total	2381 (71.3)	901 (27.0)	58 (1.74)	3340 (100)	2399 (70.9)	906 (26.8)	79 (2.3)	3384 (100)	

SCVA subgroups: Good Vision=SCVA Log MAR 0.3 or better, low vision = SCVA between Log MAR 0.3 and Log MAR 1.0 and poor vision=SCVA of Log MAR 1.0 or worse.

Table 9: A comparison between reported prevalence of AMD grades (in either eye), across age-groups and gender.

Values are percentages.

		Age, years						
	AMD score	65-69	70-74	75-79	80-84	85-90	≥90	Total
BDES	Late AMD (4a and 4b)	1.4		≥75: 7.2				1.6 (ages 43-86)
BMES	Late AMD (4a and 4b)	0.7		5.4		≥85: 18.5		1.9 (≥49 years)
TILDA	4a Males	0.3		≥75: 1.6				0.6 (in over 50 years population)
	4a Females	0.2		≥75:1.0				
	4b Males	0.2		≥75: 1.1				
	4b Females	0.2		≥75: 1.0				
EUREYE	4a Males	0.51	0.56	1.91	≥80y: 1.39			1.2
	4a Females	0.11	0.95	1.18	≥80y: 5.75			
	4b Males	0.38	1.40	2.63	≥80y: 5.56			2.3
	4b Females	0.92	1.42	2.17	≥80y: 10.50			
BEAP	4a	1.06	1.50	2.60	3.38	9.84	9.09	4.3
	4b	0.94	0.37	2.35	3.56	5.46	12.12	
	4c	0.24	0.09	0.37	0.38	0.55	0.00	
	Total	2.24	1.96	5.32	7.32	15.85	21.2	

Chapter 4: Characteristics of Geographic atrophy in an elderly UK population and the association with reticular pesudodrusen-The BEAP

4.1 Introduction

GA is the atrophic late stage of non-exudative AMD. While CNV is the most common cause of severe sight loss in AMD, GA still accounts for approximately 20% of AMD patients that are legally blind [3, 138, 139]. Recently, the treatment of nAMD has been revolutionized by the introduction of intravitreal injections of anti-VEGF. Unfortunately, there are currently no proven treatments for GA as yet, although several trials are currently underway. There is no exclusivity between GA and nAMD, with the risk factors for their development being similar. The mechanisms that result in one eye developing GA versus nAMD remains to be elucidated. GA is characterised by areas of well-defined loss of RPE and underlying choriocapillaris such that the larger choroidal vasculature becomes visible. The term was originally applied by Gass to the late manifestation of AMD, where one or more areas of atrophy develop with subsequent slow enlargement to involve the fovea[114]. For unknown reasons the spread of GA peripherally appears to be faster than the spread towards the fovea [140].

Visual loss from GA is only profound when the central subfoveal area is involved, in which case patients may develop legal blindness. However, it is

incorrect to assume that patients with foveal sparring GA will be asymptomatic. Such individuals are known to have reduced reading rates, and magnification can sometimes be detrimental as the text becomes too large to fit within their foveal-sparring scotoma, and as such visual impairment of the patient and difficulty performing daily tasks may not be adequately reflected by a simple VA recording [141] [142]. Unless future treatments are regenerative, they should preferably be applied before central foveal involvement takes place or a large scotoma develops. It has previously been reported that any treatment to stop or reduce the progression of GA would be less likely to benefit the daily visual function of individuals with a total GA size of $\geq$17.5 mm^2 [143]. As such, it is important to determine the relative frequency of GA lesions in the macula within the population, along with their distribution, distance from the fovea and area involved along with the proportion of GA that involves the fovea. This will give a clearer idea of the number of patients that could benefit from future treatments.

It is also becoming clear that GA is a more heterogeneous condition than previously reported, especially with the recent utilization of multimodal imaging for its analysis. Different GA phenotypes are being recognised such as the 'diffuse-trickling' subtype identified with the use of FAF imaging [61] which appears to be associated with RPD. Different phenotypes of GA may have different prognostic implications, but a paucity of data exists on the different phenotypic prevalence of GA within large population based studies and especially with characterisation of the peri-lesional area surrounding the GA.

There are only a limited number of publications to date that aim to subcategorise the pathological mechanisms behind atrophy of the RPE. Some authors have subcategorized atrophy as drusen-associated and neovascularization associated [144, 145]. Neovascular-associated RPE atrophy can have an appearance identical to the drusen associated GA in the absence of any active bleeding or leakage from the CNV. The RPE loss that occurs is in part at least, secondary to the fibrovascular proliferations that occur in association with the CNV. This form of RPE atrophy will not be discussed any further in this chapter.

The prevalence and incidence of GA in different populations has been previously reported [8, 14, 146, 147]. The natural history and progression of GA has also been assessed in both large scale population studies, including the BDES [148] and the BMES [149], and hospital based cohorts [115, 139, 149-153]. However, to the best of our knowledge, no detailed studies relating to GA are available from the UK population. The prevalence of GA (grade 4b AMD), measured at 2.5% in the UK population aged 65 years and older was reported in an earlier chapter of this thesis (chapter 3).

GA can have various configurations. It can take the form of a single round lesion or it can be multifocal. The phenomenon of foveal-sparing has also been described, where GA appears to enlarge outside the fovea, with only late involvement of central vision [139, 140, 151]. A subgroup of GA patients has also been described where there is coalescence of atrophic areas with the development of a 'horseshoe' or later 'ring' configuration, with atrophy surrounding a preserved

fovea [140, 151]. Reasons to explain the phenomenon of foveal sparring seen in GA remains unclear. Several authors have postulated that the unique choroidal blood flow to the fovea may be protective against atrophy [154]. Others have considered whether the high concentration of luteal pigments within the central macula may have a protective role [155]. Given the higher density of cone photoreceptors within the fovea, it has been suggested that perhaps rods have a higher vulnerability with regard to the underlying disease process [156-159].

It appears that the prediction of GA development is based predominantly on local ocular features such as drusen size or area, hypopigmentation and hyperpigmentation of the RPE rather than demographic and genetic factors [160]. In the Age-Related Eye Disease Study (AREDS) study, the average duration from drusen confluence to GA development averaged 5.9 years [115]. The AREDS group has published a severity scale that allows quantification of fundus photographic features in order to determine the risk of progression to late AMD [161]. Although this severity scale is complex and was designed as a research tool based on FP analysis by trained individuals within a reading center, it does appear to have some predictive value. An alternative and highly simplified severity scale has also been published by the AREDS group [162]. One shortcoming of the AREDS classification is its failure to take into account the presence of RPD. A strong association between RPD and advanced AMD has long been described [30]. Cohen et al noted that 24% of patients with newly diagnosed CNV had RPD present on red free or blue filter FP [35] and Schmitz-Valckenberg et al found that 62% of patients with GA had RPD identifiable with scanning laser ophthalmoscopy

imaging (SLO) [163]. In a study by Xu et al, GA progression was highly correlated to areas with RPD, compared to those areas without [164]. They also reported an almost universal presence of RPD in multilobular GA along with the observation of subsequent enlargement of individual GA lobules into areas of reticular lesions [164]. The multilobular form of GA appears far more prevalent than solitary GA in patients with RPD [55, 164]. GA progression rates are known to be higher in eyes with multilobular GA or with large initial lesion sizes [165]. It is also interesting that the fovea appears to be less vulnerable to RPD development as compared to peripheral macular areas (29). There is no literature available to suggest that, such a relationship exists in a population setting.

The present study investigated the distribution of GA in the macula in eyes included in the BEAP population cohort in order to determine several factors. Firstly, the prevalence of GA, its size, its distance from the fovea, its phenotypic characterization and visual acuities of these eyes is described. This will inform the potential treatment load for GA in the UK when treatments become available. The association between multifocal and subfoveal GA is described and the relationship of GA with RPD is explored.

4.2 Methods

The methods of BEAP, image acquisition and analysis are discussed in detail in the Methods of Chapter 2 and 3. GA was diagnosed if there was a sharply demarcated area of RPE loss that was at least 175µm in diameter within the ETDRS grid. It should be roughly round or oval in shape, with at least 2 of the

following features: scalloped edges, visible choroidal vessels that are more prominent than in the surrounding areas and well defined margins in keeping with the clarity of the fundus photograph. If any part of the area of GA was contiguous with peripapillary atrophy, then it was not graded as GA. Any eye with combined GA and contiguous nAMD was graded as the latter. All questionable lesions and all lesions that were graded as GA were scrutinised by a retinal specialist with expertise in image grading (WMA). Any differences in opinion were sent to CARF for secondary grading. Frequent sessions of simultaneous grading were performed to maintain reproducibility. If doubt existed as to whether a lesion resembled GA or other pathology such as diabetic retinopathy, pathological myopia, chorioretinitis or laser burns, then it was not graded as AMD. We retrospectively reviewed the BEAP data for all patients with GA and none had a recorded history of diabetic retinopathy or previous macular laser, making misclassifications unlikely.

All eyes identified as having GA were subsequently reviewed in greater detail and digital images analysed using the IMAGEnet 2000 program. Each eye with GA was assessed specifically for the presence or absence of RPD, which were considered present if there was a definite reticular pattern of round or oval yellow-white lesions that joined to form an ill-defined network of broad, interlacing ribbons. The FP was initially graded without digital alteration and subsequently graded with adjustment to the red-free and the blue channels of the fundus photograph using the IMAGEnet 2000 program. The area of GA was outlined on the digital FP using the draw tool within the IMAGEnet program.

Digital enhancement of the FP was allowed using the IMAGEnet program to allow improved contrast if the margin of the GA was not easy to identify in certain areas. The area was measured in square millimeters (mm^2). For each eye with GA, the shortest linear distance from the GA circumference to the presumed fovea was measured and recorded. The pattern of the GA was also categorized as either solitary or multifocal and the shape of the GA was recorded as either: round, horseshoe shaped or a foveal-sparring ring pattern. Each eye was graded as to whether the fovea was involved by the GA. The perilesional area of the GA was also sub-categorised into several descriptive groups. After independent grading of the images, LogMAR visual acuities were recorded for each eye, corrected with both current glasses and pinhole (Baylie Lovie no.4 chart). All eyes with GA had their remaining fundus graded for signs of ARM and a Rotterdam grade assigned.

Variables that may be associated with GA including smoking history, hypertension, cardiovascular disease, LOCSIII grading were evaluated.

4.3 Results

Out of the total of 3475 participants who had gradable photographs in at least one eye, a total of 85 persons were identified with GA in at least one eye, giving a prevalence in the over 65-year population of 2.5% as reported previously earlier in chapter 3. All patients were Caucasian. As shown in Table 10, a total of 130 eyes were identified with GA; 67 right eyes (2.01%) and 63 left eyes (1.86%). More females were identified with GA (48 subjects [56.5%] vs 37

subjects [43.5%]). The gender specific prevalence rates did not differ, at 2.48 % in females and 2.41% in males. At the date of examination, subject age ranged from 65 years to 96 years, with a mean age of 79.5 (SE=0.75). GA prevalence increased with age, and is shown in Table 11. A total of 29 subjects (34%) had bilateral GA, giving a prevalence of bilateral GA in the Bridlington community of 0.91% (out of a total of 3175 subjects with photos available from both eyes). Eight (8) subjects had GA in one eye and contralateral nAMD in their second eye, giving a total of 43.5% of patients with GA bilateral advanced AMD (either GA or nAMD).

The mean GA area for right and left eyes was 5.28 mm^2 and 3.61mm^2 respectively. The overall mean area was 4.51mm2 (SD5.78). A total of 8 eyes had a GA area over 17.5$mm^{2.}$ Foveal involving GA occurred in 22 right eyes (32.8%) and 19 left eyes (30.2%), with a total of 41 eyes having foveal involving GA (31.5%). The mean shortest distance to the fovea from the circumference of GA in all eyes was 460µm, while for eyes with eccentric GA it was665µm (SD 432). Multifocal GA occurred in 25 right eyes (37.3%) and 22 left eyes (34.9%). RPD were present in 11 right eyes with GA (16.4%) and 16 left eyes with GA (25.4%). There were a total of 27 eyes with GA and RPD (20.8%), with 59.3% of these eyes occurring in females. No statistically significant difference in gender specific prevalence for eyes with GA and RPD was identified, with 27.3% and 23.8% of females and males respectively having both GA and RPD (p=0.699, Pearson Chi^2). Eyes with GA and RPD were on average older than those without RPD with RPD (84.1 years [SD 5.9] vs 78.4 years [SD6.8], p=<0.05 with two-

sample t-test). A total of 47 eyes were identified with multifocal GA. The mean area of GA was similar in eyes with multifocal vs solitary GA as shown in Table 12. On average, GA eyes with RPD had larger areas of GA, and this was consistent for both right and left eyes as displayed in Table 12. The shortest linear distance from the circumference of the area of GA to the presumed fovea was similar between eyes with, and those without RPD (Table 12). Fovea involving GA was more common in eyes without RPD (35.0%) than those with (18.5%). Out of all 103 eyes with GA but no RPD, 36 (35.0%) had foveal involving GA. Only 5 of 27 (18.5%) eyes with RPD had fovea involving GA. The difference did not reach statistical significance (two tailed p=0.16, Fishers exact test). A contingency table for eyes with and without RPD versus subfoveal GA is shown Table 13. Mean VA did not appear to differ between subjects with GA and RPD vs those with GA but no RPD (Table 12).

Out of the total of 47 eyes with multifocal GA, 34 (72.3%) had no RPD present. Thirteen (13) out of the 27 eyes (48%) identified with RPD had multifocal GA. A higher prevalence of RPD occurred in eyes with multifocal GA (13 out of 47 eyes [27.7%]) compared to eyes with solitary GA (14 out of 83 eyes [16.9%]). The difference did not however reach statistical significance (p=0.18). The configuration of GA differed between eyes with RPD and those without. The majority of eyes with RPD (19/27; 70.4%) had a u-shaped area of GA or adjacent contiguous hypopigmentation. U shaped GA was an infrequent finding for eyes without RPD, with only 15 of 103 eyes having this configuration to the GA (14.6%). The difference was deemed statistically significant with a p-value of

0.001 (Fisher's exact test). Although only 48.1% of eyes (13/27) with RPD had multifocal GA, 7 out of 27 (74.1%) eyes had either multifocal GA or evidence of a significant area of peri-lesional hypopigmentation giving a U shaped area. When all eyes with subfoveal GA were excluded, the mean LogMAR BCVA was worse for eyes with GA and RPD (LogMAR 0.32, SD 0.23) than for eyes with GA but no RPD (LogMAR 0.25, SD 0.21). This difference was not statistically significant with an unpaired t-test p value of 0.25.

The majority of center involving GA (87.8%) occurs in eyes with conventional drusen only, with no RPD, while the majority of GA that occurs in eyes with RPD (81.5%) appears to be extrafoveal. Not surprisingly, RPD have a higher prevalence in extrafoveal GA (24.7%) within the BEAP population than in eyes with fovea involving GA (12.2%). The difference did not however reach a level of statistical significance, p=0.18.

70.4% of eyes with RPD and GA, had atrophy that appeared to be horseshoe (or U or ring shaped), often spreading around the fovea. Frequently, the perilesional areas continued as diffuse areas of adjacent hypopigmentation. Horseshoe shaped GA associated with RPD often had distinctive, widespread and dense clumps of hyperpigmentary changes within the center of the horseshoe shaped GA. There was also frequently a hyperpigmented border on the edge of the GA lesion. For eyes with horseshoe shaped GA, there was a high prevalence of RPD (55.9%), which was considerably greater than the prevalence of RPD in eyes with round GA (8.3%). This association was statistically significant, p=0.0001.

18 of 130 eyes with GA (13.85%) had a BCVA of LogMAR 1.0 or worse.

Only 6.9% (9 0f 130) of eyes had no or minimal morphological changes in keeping with ARM outside the area of GA. 25% (33 of 130) of eyes with GA had Rotterdam stage 3 outside the area of GA.

Table 10: GA prevalence for right eye, left eye, worse eye and best eye.

Data is number (percentage)

Grade	Right eye	Left eye	Worse eye	Best eye
GA	67 (2.01)	63 (1.86)	85 (2.45)	48 (1.38)
Total	3340 (100)	3384 (100)	3475 (100)	3475 (100)

Table 11: Age specific prevalence of GA for right eyes and left eyes and worse eyes.

Data is number (percentage)

	65-69	70-74	75-79	80-84	85-90	≥90	Total
Right Eye	6 (0.7)	14 (1.29)	15 (1.82)	14 (2.57)	16 (8.33)	2 (5.88)	67 (1.89)
Left Eye	7 (0.82)	9 (0.83)	17 (2.06)	15 (2.76)	12 (6.25)	3 (8.82)	63 (1.78)
Worse Eye	10 (1.18)	16 (1.50)	21 (2.60)	19 (3.56)	18 (9.84)	3 (9.09)	87 (2.50)

Table 12: Summary of GA parameters for right and left eyes.

Units are area, mm^2 (SD), linear distance, mm (SD).

Mean value	Right eye	Left eye
GA area Multifocal GA	4.9 (6.52)	3.07 (2.81)
GA area solitary GA	5.49 (7.71)	4.03 (3.38)
GA area for eyes with RPD	5.46 (7.27)	5.27 (6.96)
GA area for eyes with no RPD	5.12 (7.34)	3.28 (4.73)
Shortest linear distance from GA circumference to fovea in eyes with RPD	0.44 (0.24)	0.53 (0.40)
Shortest linear distance from GA circumference to fovea in eyes with no RPD	0.43 (0.55)	0.48 (0.51)
Mean LogMAR BCVA for eyes with GA and RPD (and VA better than CF)	0.35 (0.25) With an addition of 1/11 eyes (9.1%) having CF VA or worse	0.37 (0.30) With an addition of 2/14 eyes (14.3%) having CF VA or worse
Mean LogMAR VA for eyes with GA and no RPD (and VA better than CF)	0.36 (0.28) With an addition of 9/56 eyes (16.1%) having CF VA or worse (p=0.88)	0.31 (0.24) With an addition of 6/47 eyes (12.8%) having CF VA or worse (p=0.47)

Table 13: Contingency table comparing subfoveal GA vs extrafoveal GA for eyes with and without RPD.

Data is number (%). Two tailed p-value (Fisher's test) =0.16.

	RPD	No RPD	Total
Subfoveal GA	5 (18.5)	36 (35.0)	41 (31.5)
Exrafoveal GA	22 (81.5)	67 (65.0)	89 (68.5)
Total	27 (100)	103 (100)	130 (100)

Table 14: A contingency table comparing multifocal GA vs solitary GA for eyes with and without RPD.

Data is number (%).Two tailed p-value (Fisher's test) =0.18.

	RPD	No RPD	Total
Multifocal GA	13 (48.1)	34 (33.0)	47 (36.2)
Solitary GA	14 (51.9)	69 (67.0)	83 (63.8)
Total	27 (100)	103 (100)	130 (100)

Table 15: Contingency table comparing U-shaped/horseshoe GA with round GA for eyes with and without RPD.

Data is number (%). Two-tailed p-value=0.001 (Fisher's exact test).

	RPD	No RPD	Total
U shaped GA	19 (70.4)	15 (17.0)	34 (26.2)
Round GA	8 (29.6)	88 (85.4)	96 (73.8)
Total	27 (100)	103 (100)	130 (100)

4.4 Discussion

Several studies have previously reported the prevalence and incidence of GA from different populations [8, 94, 108, 146, 148, 150, 166]. In the UK, prevalence figures were previously based on registration of visual impairment [132], a population study of individuals with reduced vision in the over 75 year olds [24] and a recent review by Owen et al [25], who estimated that GA prevalence rates were 2.6% (95% CrI [credible interval] 1.8% to 3.7%) and 6.7% (95% CrI 4.6% to 9.6%) in the over 65 year, and over 80 year population respectively. Data from robust population studies are limited. This chapter provides an updated and detailed report on GA prevalence in the UK, using data acquired from the standardized use of digital FP and recognized grading methods. It represents the largest population based study to date in a UK over 65-year population. The findings

indicate that GA prevalence increases significantly with age, from 1.18% in the 65-69 year age group, reaching a maximum of 9.84% in the 85-90 year age group. The prevalence did not vary between genders.

In the present study, bilateral GA occurred in 34% of subjects; giving a community prevalence of bilateral GA in the Bridlington over 65 years population of 0.91%. This rate is lower than some previous reports, suggesting bilateral disease can occur in up to 48% to 65% of prevalent cases [145, 147, 167, 168]. One consideration is that many previous studies are hospital based and the patient population will likely have cohorts consisting of more advanced and symptomatic disease. Sunness et al reported in a natural history study that 59% of patients had bilateral GA [139]. In the BMES, Joachim et al report central involvement was evident in 57% of eyes with GA at first presentation [147] and in 47% of incident lesions. Klein et al reported bilateral GA was found in 23% of GA subjects within the BDES, which is very similar to our reported figure. This has major implication on prognosis as it poses a significant potential for future loss of vision in both eyes and it highlights the high degree of concordance and tendency for bilateral involvement in eyes with GA in a community setting.

We can report that monocular legal blindness from GA occurred in 13.9% of GA eyes within the Bridlington over 65 year population. This is lower than the 23% reported by Sunness et al in a hospital based cohort of GA subjects at baseline [139]. We acknowledge that this may be an underestimation given individuals who were known to be blind or partially sighted were excluded from the BEAP at recruitment.

It is clear from this study that GA is a heterogeneous condition with a large spectrum within the community. There are some features however, that appear more frequently. The majority of eyes with GA in the Bridlington community (63.8%) appear to have a single area of atrophy, with only a minority (36.2%) having a multifocal configuration. This figure is very similar to the 78% reported for solitary GA from the CAPT study [169]. The CAPT study also reported the finding that the majority of incident GA consists of small areas of parafoveal atrophy, with a mean distance from the most proximal edge of GA to the fovea of 430μm (SD 400). This study reports similar findings, with the mean distance in all eyes being 460 μm. In the BDES, the rate of solitary GA, at baseline when GA was first identified, was 45%. The reasons for the lower rate may reflect the younger population at enrollment in the BDES (43 to 84 years), or may represent the addition of a third grading category. In the BDES, Klein et al divided GA into 3 configurations based on the appearance of atrophy in the fundus photographs. Their first was classic, and referred to a single, usually round area of GA. The second was multifocal, indicating two or more separate areas of GA. The third, referred to as 'merged', was reported to represent multifocal lesions that had grown together into one or more large irregular lesion, often with satellite areas of atrophy projecting off the side. In this report, a single large area of GA with a projection was recorded as solitary GA. It was felt that drawing assumptions on the origin of large areas of GA, in terms of whether they enlarged concentrically from a small round GA or from the union

of multifocal areas was not possible. Although Klein et al [148] do not report the number of solitary 'merged' areas of GA, it may be that if this category was combined with their solitary GA, the figure may be more comparable to our reported prevalence of single GA. The lower rate of incident multifocal GA at presentation in the CAPT study (22%) when compared to the BEAP study (36%) is not a surprising finding given the lower mean age at enrollment when comparing the two studies (70.6 years CAPT study, vs 73.4 years BEAP). Another possible explanation for the difference, is that the CAPT population, with multiple (≥10), bilateral and large (≥125) drusen at enrollment may not be representative of the community AMD population, and will include primarily early incident GA lesions.

Multifocal GA is known to be a predictor for a greater rate of progression [148]. As such, it is important to know the occurrence of such configuration of GA in the population in order to inform health planning. In the BDES, eyes with multifocal GA had the greatest mean increase in GA area (12.0mm^2), with areas of solitary GA having the least (2.2mm^2) [148]. This finding was repeated in the BMES [147]. Multifocal GA was also most likely to progress to involve the fovea [148]. In this study, eyes with multifocal GA did not, on average, have a larger area of GA when compared to eyes with solitary GA. This finding is the same as that in the BDES. One explanation for this is that perhaps there are sub-phenotypes of multifocal GA and some progress more rapidly. Once united, they would be categorized as a single lesion with a large area. Slowly progressive single GA, and more rapidly progressive multifocal GA are indeed indistinguishable when

large and at the end stage of their progression. Perhaps a better sub classification is required.

The findings in this report are in keeping with the previous notion that GA lesions are predominantly small and parafoveal, and have gradual enlargement [169]. A significant proportion of eyes (31.5%) in this community based population have fovea involving GA. This is an important finding given GA is only associated with a significant reduction in VA when lesions encroach or involve the fovea center. As expected, this is higher but not dissimilar to the findings in the Complications of Age-Related Macular Degeneration Prevention Trial (CAPT) study [169], where 20% of incident GA lesions were subfoveal at presentation. A similar proportion of central GA was reported from the AREDS group (24%). At baseline in the BDES 50% of eyes with GA had central involvement [148]. In the BMES there was a higher frequency of central fovea involving GA at presentation (57%), using FP [166]. Fleckenstein et al report foveal sparing in 58% of eyes with a particular phenotype of GA (classified with the use of FAF imaging) referred to as the diffuse trickling phenotype [60, 61]. It highlights the heterogeneity of GA, with some lesions involving the fovea early on while others enlarge significantly with foveal preservation. In the CAPT study, it was reported that 70% of cases of incident subfoveal GA occurred secondary to regression of a drusenoid PED. They reported that the presence of a drusenoid PED results in a distinct disease process that places them at substantially higher risk, not only for GA, but also GA that has the potential for significantly reduced vison secondary to foveal involvement [169]. Other groups have reported the

finding that drusenoid PED's are a particular risk for a high rate of progression to AMD, in particular for GA with foveal involvement [170, 171]. In the BDES, when pure GA was first observed, it involved the foveal center in 50% of cases [148].

In the BDES at initial identification, the overall mean area of GA was 4.6mm^2 (SD 6.00). This parameter did however vary according to GA configuration, being similar for classic single GA (2.72mm^2 [SD 4.54]) and multifocal GA (2.02mm^2 [SD 1.91]) but significantly larger for merged GA (8.21mm^2 [SD7.14]). The findings from the BMES are similar (5mm^2) for pure GA eyes at baseline [147]. The mean area of GA in the Bridlington UK population appears very similar (4.51mm^2). In the CAPT study, the overall mean area of GA per eye was 0.57mm^2 (SD 0.75). This considerably smaller size of lesions, reflects the early incident GA recorded in the CAPT population. Like in the BDES, a difference in the mean area of GA for solitary or multifocal configurations could not be demonstrated from this present study.

Previous population based and natural history studies [107, 108, 148, 151, 172] have failed to consistently reveal any systemic factors associated with an increased occurrence of GA or an increased rate of progression. It is not surprising, given the small numbers of GA involved in some studies, and the associated low power, that studies may fail to identify risk factors. Only age and smoking have consistently been related to GA in systematic reviews [173-175]. Local ocular factors have, however, been consistently identified as the most important risk factors across a wide range of studies. Sunness et al, in a clinic based natural history study demonstrated that the amount of enlargement of GA increased with

larger initial areas of GA [151]. After 10 years of follow up in the BDES, eyes with soft indistinct drusen or retinal pigmentary abnormalities at baseline, were more likely to develop AMD at follow-up than those without (15.1% vs 0.4% and 20.0% vs 0.8%, respectively). The overall 5-year incidence of GA was 0.3%, but this was more than 3 times greater (1%) in individuals with large drusen ≥125μm, and higher still (8%) for individuals with very large drusen ≥250 μm at baseline. In the Rotterdam Study, the incidence of AMD was strongly associated with the stage of age ARM at baseline, with the most important predictor for progression being more than 10% of the macular area being covered by drusen (odds ratio [OR] 5.7, 95% confidence interval [CI] 2.9-11.3. [108]. Other independent prognostic features for progression were the presence of hypopigmentation (OR 4.0, CI 2.5-6.4) and hyperpigmentation (OR 3.4, CI 2.1-5.4). In the CAPT study, the majority of GA (84%) occurred in areas previously occupied by drusen; 35% being secondary to regression of soft confluent drusen and 35% secondary to dresenoid PED regression (CAPT).

One neglected area of risk stratification is the influence of RPD in eyes with GA, for which there is a sparse data available from population based studies, with only two reporting their prevalence and incidence [37, 147]. It is known that RPD have a relatively low overall community prevalence, being 0.7% in the BDES population [37]). After initial description, their association with advanced AMD was confirmed by various groups [30, 37, 55, 61, 176]. Cohen et al utilised red free (RF) and blue-filter photographs to demonstrate a high prevalence of 24% of RPD among patients with AMD [35]. They have been shown to confer an increased risk

of progression to GA, in addition to drusen and pigmentary changes (Hazard ratio [HR], 4.93, p=0.042) [177]. The results from the Bridlington population confirm an increased prevalence of RPD in eyes with GA of 20.8% from FP grading.

Unlike conventional drusen, RPD are more common in woman [30, 37, 178]. Given the association of RPD with GA, and the female preponderance of RPD, it is surprising that in this study, or others, the gender specific prevalence of GA does not differ. For some reason, the increased prevalence of RPD in women does not appear to translate to a higher frequency of GA. The reasons for this remain unclear. RPD are known to increase in prevalence with age, in the BDES increasing in prevalence from 0.4% in the 43-54 year age group, to 6.6% in the 75-86 year age group [37]. The BEAP study confirms that eyes with GA and RPD, were on average older than those without RPD.

RPD are known to be associated with nAMD [30, 179]. In the AREDS their presence was associated with a 5-year incidence of nAMD of 29%. In the BDES their presence at baseline was associated with the highest 15-year cumulative incidence of nAMD (20% as compared to 10% for a soft indistinct drusen) [37]. Our research team have previously demonstrated that RPD have a prevalence of 22% in eyes with newly presenting nAMD [179]. This figure is also similar to the prevalence of RPD in patients with PPCNV (30%, Chapter 6). In a study by Xu et al [164], it was reported that GA progression was highly correlated into areas of RPD, compared to areas without. RPD have also been reported as an almost universal finding among eyes with multilobular GA, and enlargement of

individual lobules has been documented in areas with RPD [164]. In the present study, multifocal GA occurred more commonly in eyes without identifiable RPD on FP. This is not surprising, given that conventional drusen are far more prevalent than RPD, and drusen regression and drusenoid PEDs are established causes of GA. The equal occurrence of RPD in multifocal and solitary GA in the present study is in contrast to the findings of some previous reports where multifocal GA was reported to be far more common than solitary GA in eyes with RPD [55, 164]. These studies are however hospital based and therefore may underrepresent early, unilateral and asymptomatic GA eyes. RPD did have a higher prevalence in eyes with multifocal GA when compared to eyes with solitary GA, however this difference did not reach a level of statistical significance. Similar to the findings of absence of RPD in 71.4% of cases reported by Marsiglia et al [165] most eyes that had solitary GA in this present study did not have RPD.

RPD are a more frequent finding in the superior macular area, specifically superotemporally between the upper edge of the fovea and the superior vascular arcade [30, 37, 49, 140, 180, 181]. In a cohort of AMD patients, Sarks et al [182] demonstrated a relative sparring of the fovea in eyes with RPD, in contrast to eyes with conventional drusen, which had a predilection for the fovea. Steinberg et al [183] demonstrated foveal sparing of RPD in 54% of eyes with early and intermediate AMD. These finding are supported by histological reports that demonstrate a paucity of SDD at the fovea, but rather abundance in the perifoveal regions [184].

A clear finding from this study is that GA configuration differs between eyes with RPD and those without, with a horseshoe shaped GA being far more common in eyes with RPD than those without. This finding is not surprising given the previously reported strong spatiotemporal association between RPD and GA progression in the setting of dry AMD [165]. For both right and left eyes, the mean area of GA was larger for eyes with RPD than those without. It is suggested that horseshoe shaped GA in eyes with RPD should be considered as a separate GA phenotype. This study draws comparisons to the diffuse trickling phenotype described by others using FAF imaging and point out similarities with this phenotype that are described here using FP. More research is required to confirm if indeed it is the same GA phenotype and to explore its natural progression. This confirmation is important as it has been demonstrated that the diffuse-trickling phenotype has the highest progression rate over time (median, 3.02mm^2/year). It is highlighted that the term multifocal GA is non-specific and could represent the pathological process of two large regressed indistinct drusen, to several areas of GA occurring in a horseshoe pattern and associated with RPD. It is acknowledged that some eyes will have a combination of the two and it is emphasised that individuals with no identifiable RPD could still gave a GA configuration that is horseshoe shaped with foveal preservation. An important distinguishing feature however remains the lack of central diffuse and prominent RPE changes. It is suggested that the term horseshoe shaped GA with RPD may be a more appropriate term for use in future studies as it may

subcategorise GA phenotype more appropriately according to the likely aetiological drusen phenotype that may subsequently impact progression.

One may expect eyes with RPD and GA to have a better visual prognosis, given their tendency for foveal sparing. This was not the case with both groups having similar numbers of eyes with VA reduced to CF or worse. It is hypothesized that despite the greater tendency for RPD associated GA to be foveal sparing, the VA is still reduced secondary to the significant pigmentary epitheliopathy that often involves the central macula before the development of definite center involving GA in RPD patients. After excluding all eyes with subfoveal GA, mean BCVA was worse for eyes with GA and RPD (0.32, SD 0.23) than for eyes with GA but no RPD (0.25, SD 0.21). This difference did not reach statistical significance however.

The main limitation of this study is the utilization of only for identifying and measuring GA lesions. It has been noted by some that areas of GA, especially when small, or in a pale fundus, can be difficult to identify and accurate delineation of borders can be problematic [185]. FP can have reduced resolution in the presence of media opacities. It uses light in the visible spectrum to capture the fundus image, and identifies boundaries of GA by their colour difference. It can however be challenging to distinguish GA that has a pale yellowish colour from depigmented RPE or confluent drusen that also appear yellow [186]. The reproducibility of FP can be less when compared to other methodologies such as FAF [187].

FAF is the current gold standard for the assessment of GA, often used in conjunction with FP. In this technique the fluorescence originates from lipofuscin, naturally occurring within RPE cells [188]. In a healthy eye, the pattern of autofluorescence is uniformly distributed, diminishing towards the fovea secondary to absorption by macular pigments. In areas of GA, the lipofuscin containing cells within the RPE are absent and so distinct, dark areas are observed representing a loss of the fluorescent signal. Because of the better contrast as compared to colour differences seen with FP, FAF offers better delineation of borders and identification of early GA [189]. FAF also provides further diagnostic information currently not provided by FP [190]. In the junctional zone of GA, hyperflurescent areas are often observed that are believed to represent regions of RPE cells that are stressed and more likely to become atrophic [191]. Further to this finding is the report that this junctional abnormal autoflurescence predicts the risk of GA progression [192] and specific patterns predict the rate of progression [193, 194]. The predictive power of FAF would make its inclusion in any future study on GA prevalence/incidence paramount.

It can also be difficult in parafoveal GA to delineate foveal sparing. Ideally, the use of multimodal imaging, particularly high contrast imaging modalities such as FFA or FAF would have been preferred for lesion circumference delineation. Infrared reflectance imaging (IR) would give a clear well-demarcated bright zone of hyper-reflectance and has been shown to be an excellent imaging modality for GA patients, offering further information and new/novel signs like 'ghost drusen' that are simply not possible with FP alone [195, 196].

The use of SD-OCT would have been useful to allow confirmation of fovea involvement, as well as providing information on structural integrity of retinal layers. Multimodal imaging would have also allowed the more sensitive detection of RPD and allow a more detailed assessment of GA and perilesional areas that may have revealed more important information about different phenotypic variations of GA. There is a clear need for future studies to make use of recent developments in imaging technologies. The use of multimodal imaging in a population based study would allow much more sophisticated phenotyping of AMD and other retinal diseases, and any future or follow up studies would utilize SD-OCT and IR. Exclusion of individuals living within the Bridlington area at the time of the study and who were known to be registered as blind or visually impaired is another limitation as it may have excluded individuals with the most advanced bilateral GA or other causes of visual loss in the contralateral eye.

Another limitation is the fact that within this population there will be several GA phenotypes that will be at different stages of their evolution. This will make comparison difficult and prospective studies and incidence studies are required to draw information regarding aetiology.

Further research is required in particular to assess whether the foveal sparing horseshoe shaped GA with RPD and the associated central pigment epitheliopathy results in a more significant visual impairment when considering daily living tasks such as reading speed. It is acknowledged that in such patients

with circumferentially spreading GA with foveal preservation, BCVA may not represent visual dysfunction.

In summary, GA has a prevalence of 2.5% for the worse eye in the over 65 year Bridlington population; increasing with age to reach a maximum of 9.84% for those aged 85 to 90 years. Bilateral GA is not an infrequent finding, occurring in 34% of subjects with known GA. In the mainstay it is largely an eccentric condition, with only 31.5% of involved eyes having subfoveal GA. The majority of GA appears to occur in the perifoveal region; particularly that associated with conventional drusen.

There is a clear association between GA phenotype and the drusen phenotype identified within the remaining fundus. RPD are in the mainstay associated with horseshoe shaped GA, with a high prevalence of 55.9% within this group. The prevalence of RPD in eyes with round GA is considerably lower (8.3%). There is a suggestion that GA eyes with RPD may have larger areas of atrophy and are less likely to have foveal involvement. However, the mean VA remains the same secondary to a diffuse, central retinal pigment epitheliopathy that appears to be associated with RPD horseshoe shaped GA.

GA is a heterogeneous condition and there remains a need to better understand and correlate its incidence and rate of progression to different drusen phenotypes and associated GA configurations. Only a minority eyes with GA in the community have large areas of atrophy over 17.5mm^2 with a mean area of 4.51mm^2. This study is the first to describe in detail the features of GA within a UK population. The information will be useful in the design of future

clinical trials that evaluate the development or progression of GA as well as modelling the impact of GA on sight loss in the UK.

Figure 2: Colour fundus photograph of typical horseshoe shaped GA with foveal preservation.

RPD are most prominent superotemporally, and an extension of the GA can be seen in that direction. The preserved fovea and surrounding retina, although not affected by the GA, is affected by diffuse and widespread hyperpigmentary and hypopigmentary changes. The circumference of the GA in these is also frequently affected by a cuff of hyperpigmentary changes.

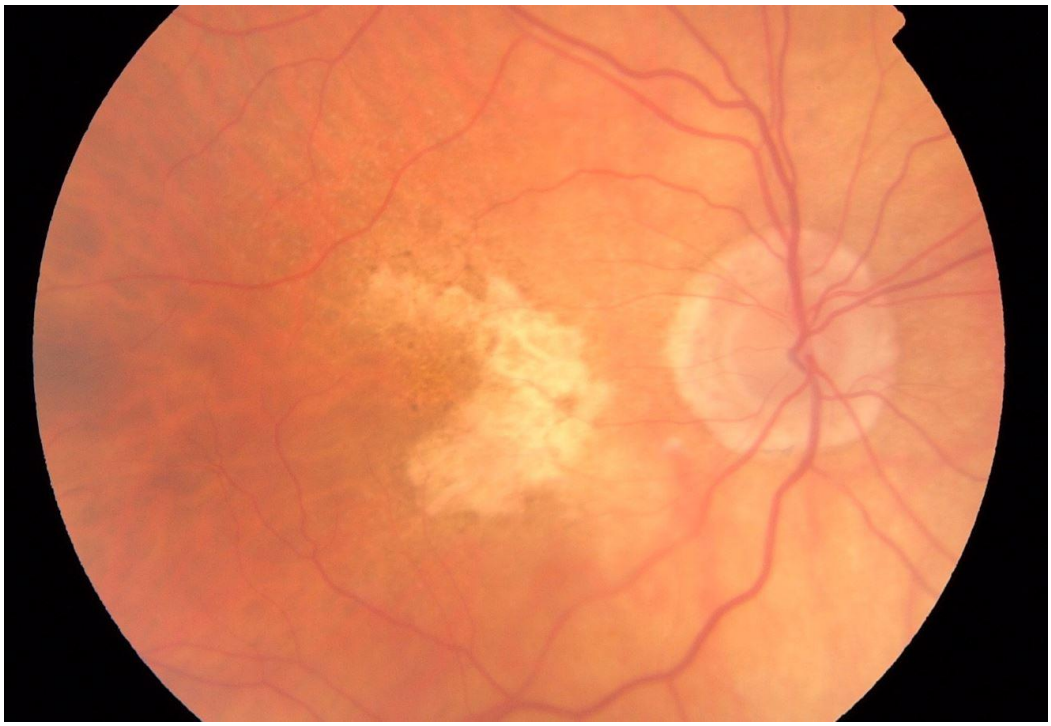


Figure 3: Colour fundus photograph showing small multifocal GA.

These small areas of early GA are typical of those that occur from drusen regression. The GA is round and well defined. They are often central or paracentral and the perilesional area often show a clear demarcation. There are no marked widespread pigmentary changes.

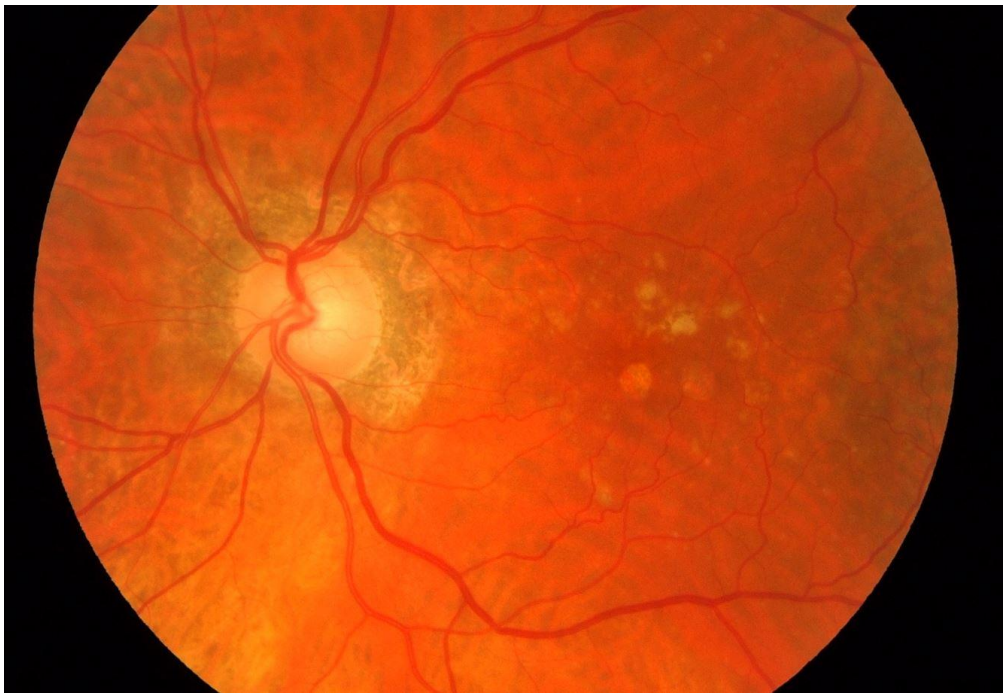
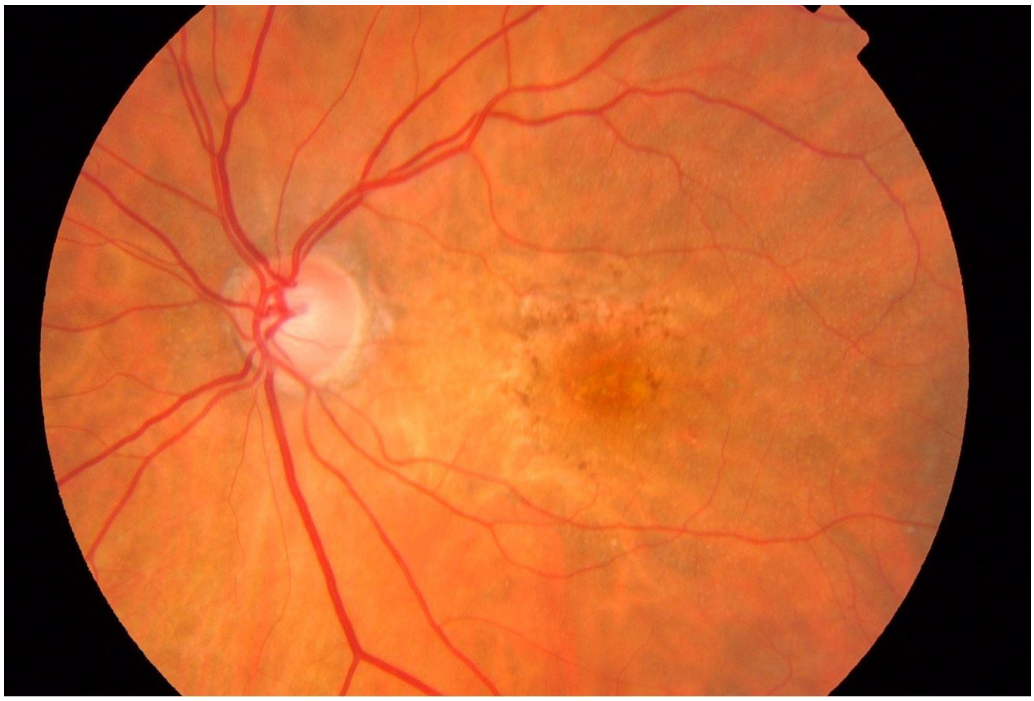


Figure 4: Colour fundus photograph of RPD with horseshoe shaped pigmentary change

RPD are clearly visible. This eye does not yet have GA but there is extensive hypopigmentation that is developing in a horseshoe pattern with foveal preservation. Widespread central hyperpigmentary changes are visible.



Chapter 5: Prevalence of RPD in an elderly UK population

5.1 Introduction

In 1990 Mimoun et al first described a yellow reticular pattern in the macula of patients with AMD [29]. They were initially described as 'les pseudodrusens visibles en lumiere bleue', or 'pseudodrusen visible on blue light' because of their enhanced visibility with blue light fundus photography. One year later, in the WARMGS, Klein et al classified them as a type of soft drusen [27]. However, these are not specifically included in the International Classification System of AMD [43]. Arnold et al reported the presence of yellow interlacing networks between 125-250μm in width and called them RPD [30]. On the basis of their findings from one histological specimen which did not have a neural retina attached, they speculated that reticular macular lesions result from poor choroidal perfusion and demonstrated progressive fibrosis in the choroidal stroma between a reduced number of large choroidal veins [30]. They hypothesised that RPD were located below the RPE and demonstrated that they increased the risk of AMD [30]. These authors suggested that the reticular pattern seen on red free photography was secondary to the fibrosis of the choroidal stroma seen between the large veins [30]. In 2006, Smith et al correlated RPD as seen on fundus photography (FP) with the reticular pattern seen on FAF imaging [34]. Later in 2009 Smith et al investigated RPD with various imaging modalities including the SLO, IR and indocyanine green angiography (ICG). They proposed 'reticular macular disease' (RMD) as a phenotypic entity in the classification of

AMD, with RPD being the term used to describe en face photographic features of RMD [178]. In an analysis with the SD-OCT in 2010, Zweifel et al recognised these RPD lesions were localised between the RPE and the inner segment/outer segment boundary (now referred to as the 'inner segment ellipsoid band' on OCT imaging, contrary to Arnold's histological report. Zweifel referred to them as SDD [44]. This study compared the reticular pattern above the RPE, as seen with SD-OCT, with the histological re-examination of SDD in three eyes previously studied by Rudolf et al [45]. Using the technique of differential interference contrast microscopy, this histological study demonstrated refractile material that corresponded to the SDD seen on OCT [44]. However, these eyes did not have a pre-mortem diagnosis of RMD and blue light was not used to demonstrate a macroscopic reticular pattern [44]. Sohrab et al later reported using multimodal imaging that the reticular pattern did not correlate with SDD seen on SD-OCT [48]. Using en face high density SD-OCT and point to point correlation, they reported that reticular lesions abutted large choroidal vessels, suggesting that they may result from choroidal changes. Querques et al and Alten et al also co-localised reticular lesions/SDD to within the intervascular choroidal stroma, providing further support for a choroidal vascular origin for reticular lesions [47, 62].

Further histopathological studies have found the composition of reticular lesions to be similar, but not identical, to that of soft drusen (apolipoprotein E, esterified and unesterified cholesterol, complement factor H, membranous

debris and vitronectin) [45, 184]. The reticular lesions, however, appear to have a higher concentration of unesterified cholesterol [197].

Since the initial reports using FP, it is now recognised that with multimodal imaging RPD have several morphological features. Their distribution can also vary considerably [198, 199]. Lee et al report that they can range from a few discrete deposits that are localised to the superior macular area, to others that have numerous deposits in all four quadrants. Morphology can vary from round to oval, well defined deposits that form a reticular pattern, to confluent deposits with surrounding branching lesions [182, 198]. Questions remain as to whether all eyes with the localised discrete pattern eventually progress to the confluent pattern and whether there are different risk stratifications for each of the morphological types. Lee et al demonstrated RPD with a diffuse distribution showed a confluent morphological pattern and the highest prevalence of late AMD [198].

There is no detailed population based report of prevalence of RPD within a UK population which is surprising given their importance and strong association with AMD. To the best of the knowledge of the author, only three population based studies have reported in detail the community prevalence and/or incidence of RPD [37, 58, 147]. BDES reported a prevalence of 0.7% at baseline in a 43-86 year-old population, with a 15-year cumulative incidence of 3% [37]. The Melbourne Collaborative Cohort Study (MCCS) reported a prevalence of 0.4% [58], and the BMES a 2% 5-year incidence [149], and a 15-year cumulative incidence of RPD as 4.0% [147]. None of these studies report using digital enhancement of

photographs to the red-free channel of the FP. This chapter reports the epidemiology of RPD within an elderly UK population and explores the associated risk factors for RPD. We report the topographical distribution of RPD along with a report of community prevalence of different morphological types of RPD as seen with FP, which to date remains unreported from a cross sectional population based study.

5.2 Methods

The methods of BEAP, image acquisition and analysis are discussed in detail in the Methods of Chapter. Examination and grading of each photograph was performed in accordance with the International Classification System of AMD (Bird et al) in which an ETDRS grid was superimposed onto digital images, centered at the fovea, dividing the macular area into 9 subfields, consisting of a central subfield, an inner circle (made up of 4 inner subfields), and an outer circle (made up of 4 outer subfields).

The FP was initially graded without digital alteration and subsequently graded with adjustment to the red-free and the blue channels of the fundus photograph using the IMAGEnet 2000 program. To do this within the viewing module, 'Utilities' was selected and out of the three principle colour channels, the red component was minimised and the subsequent enhanced image compared to the first. All images within the BEAP database were analysed in this way, even if initial review did not identify RPD. The authors acknowledge that there is no validated study that demonstrates that digital enhancement of

the FP to the blue channel is directly comparable to the use of photographic filters [200].

Each eye was assessed specifically for the presence or absence of RPD, which were considered present if there was a definite reticular pattern of round or oval yellow-white lesions that joined to form an ill-defined network of broad, interlacing ribbons. A diagnosis of RPD was made if there were only round or oval lesions, with no interlacing or confluent pattern evident, if the lesions were regularly spaced forming a reticular pattern, and had a significant uniformity in size and colour, and were more clearly visible with enhancement of the digital photograph (to the red free channel). Only if the grading ophthalmologist was definite that these lesions were not regularly arranged conventional drusen was this grade of RPD made.

All questionable RPD lesions were scrutinised by a retinal specialist with expertise in image grading (WMA). Any differences in opinion were sent to CARF for secondary grading to adjudicate the difference. Frequent sessions of simultaneous grading were performed to maintain reproducibility. All images were graded for conventional and RPD on a subfield-by subfield basis, and the presence of RPD was also recorded outside the grid and also nasal to the optic disc.

All eyes identified as having RPD were subsequently re-evaluated (re-graded) and the presence of RPD confirmed. The topographical distribution of RPD within each of the 9 subfields was recorded. Each eye with RPD was assessed specifically, for the presence of conventional drusen, hyperpigmentation and

hypopigmentation within the ETDRS grid. All RPD eyes were subcategorised for the morphological characteristics of RPD. Dot RPD were defined as discrete dots, often paler or 'whiter' in colour than conventional drusen that may be confluent. They had to form a regular pattern. Ribbon RPD was recorded if there were interlocking ribbons that could be confluent. If both RPD types were present, either superimposed on each other within the same area of the fundus, or distributed in separate areas, then a 'mixed RPD' phenotype was recorded. After independent grading of the images, LogMAR visual acuities were recorded for each eye, corrected with both current glasses and pinhole (Baylie Lovie no.4 chart).

As part of the original protocol, at attendance, all individuals were questioned specifically if they were satisfied with their current vision.

5.3 Results

Out of a total of 3476 Caucasian subjects that had gradable photographs in at least one eye, RPD were present in 281 eyes of 176 individuals, giving a prevalence in the over 65-year age group of 5.06% for either eye, including only eyes. Out of a total of 281 eyes, 44 (15.7%) were only detectable with confidence after digital enhancement of the FP to the red-free channel. RPD frequency was almost identical for right (n=140, 4.18%) and left (n=141, 4.16%) eyes. Mean age for subjects with RPD was 81.1 years (SD 6.01). Out of the 3255 subjects that had gradable photographs in both eyes, 105 (76.6%) had bilateral RPD. A total of 114 subjects identified with RPD were female (64.8%). Females had a higher gender specific prevalence rate when compared to males (5.9% vs

4.0%), as shown in Table 16. The difference reached a level of statistical significance with a Pearson Chi^2 test, p=0.014). The prevalence of RPD increased significantly with age, from 1.18% in the 65-69-year age-group, and reaching a maximum of 27.27% in the over 90-year age-group. The age specific RPD prevalence is shown in Table 17.

Of the 281 eyes with RPD, 102 eyes (36.3%) had both ribbon and dot RPD, 52 (18.5%) had only dot RPD, while 103 eyes (36.7%) had only ribbon RPD present. Using a logistic regression model with RPD as a dependent variable, RPD were associated with increasing age (OR 1.18, 95% CI 1.15-1.21, p=0.000), female gender (OR 1.52, 95% CI 1.09-2.13, p=0.014), and a history of diabetes mellitus (DM) (OR 1.97, CI 1.20-3.17, p=0.005). A history of treatment for hypertension (OR 0.64, CI 0.46-0.90, p=0.009) and previous cerebrovascular accident were not associated with RPD (OR 1.00, CI 0.59-1.69, p=1.00). RPD had a high prevalence in eyes with advanced AMD as shown in Table 18.

When present, RPD were located outside the ETDRS grid 88.2% and 88.5% of the time, for right and left eyes respectively. When present within the ETDRS grid, they were most commonly found in the outer superior subfield, 91.9% and 87.8%, for the right and left eyes respectively. RPD were found in the central grid in only 12.1-14.3% of right and left eyes. The fundus in most eyes with RPD had a tessellated appearance (Figure 5).

The patterns and appearances of RPD are heterogeneous. They appear to change according to the region of macula involved. On occasions, as RPD encroach the central subfields they can take on the morphology of dot RPD. The

closer to the fovea, the most subtle and small these lesions become. RPD involving the central subfield on FP are present, and can have a particularly distinct appearance that the author referred to as 'ultrafine RPD', as shown in Figure 6. The appearance is of an exaggerated central stippling that may form part of a continuum of the central stippled pigmented epitheliopathy often seen in RPD with a foveal sparring GA (Figure 7). The topographic distribution of RPD is shown in Figure 8 and Figure 9.

The frequency of co-morbid conventional drusen of >125µm, hyperpigmentary and hypopigmentary RPE changes, and the frequency of eyes with advanced AMD for right and left eyes is shown in Table 18. Out of a total of 158 subjects with grade 4 AMD in at least 1 eye, 41 subjects (25.9%) had RPD in at least one eye, with eyes with Grade 4c (PPCNV) having the highest prevalence or RPD. Out of a total of 281 eyes with RPD, only 36 eyes (12.8%) had no or minimal evidence of ARM (Rotterdam grade 0a/0b) within the ETDRS grid. Approximately 50% of eyes had conventional drusen greater than 125µm in size.

The relationship between subject satisfaction with vision and RPD was explored. When controlling for age, there appears to be an association with visual dissatisfaction and the presence or RPD (OR estimate using Mantel-Haenszel 0.63, 95% CI 0.45-0.88, p=0.007), as shown in Table 19.

Table 16: Gender specific prevalence of RPD.

Data are number (percentage), with only eye patients excluded. [95% CI]

Gender	RPD absent	RPD present	Total
Female	1826 (94.12) [92.98-95.09]	114 (5.88) [4.91-7.02]	1940 (100)
Male	1474 (95.96) [94.85-96.85]	62 (4.04) [3.15-5.15]	1536 (100)
Total	3300 (94.94)	176 (5.06) [4.38-5.84]	3476 (100)

Table 17: Age specific prevalence of RPD.

Data are number (percentage). [95% CI]

RPD	65-69	70-74	75-80	80-84	85-90	90 and over	Total
No	838 (98.82) [97.81-99.39]	1050 (98.22) [97.22-98.88]	766 (94.80) [93.03-96.15]	479 (89.70) [86.82-92.02]	143 (77.72) [71.15-83.15]	24 (72.73) [55.61-85.10]	3300 (94.94)
Yes	10 (1.18) [0.61-2.19]	19 (1.78) [1.12-2.78]	42 (5.20) [3.85-6.97]	55 (10.30) [7.98-13.18]	41 (22.28) [16.85-28.85]	9 (27.27) [14.9-44.39]	176 (5.06) [4.38-5.84]
Total	848 (100)	1069 (100)	808 (100)	534 (100)	184 (100)	33 (100)	3476 (100)

Table 18: Comorbidity of RPD with conventional drusen, RPE pigmentary changes and AMD.

Data are numbers (percentage)

Present within ETDRS grid	Right Eye	Left eye
Conventional drusen over 125µm	69 (50.37)	74 (53.24)
RPE hyperpigmentation	10 (7.41)	16 (11.51)
RPE hypopigmentation	2 (1.48)	4 (2.88)
Both hyper/hypopigmentation	28 (20.74)	34 (24.46)
GA	10 (15.15)	15 (23.81)
nAMD	7 (18.42)	7 (17.50)
PPCNV	2 (40.00)	1 (25.00)

Table 19: An exploration of satisfaction with vision.

	Odds Ratio	Standard Error	P value	95% Confidence interval
RPD	0.666	0.115	0.019	0.474-0.935
Age at examination	0.968	0.007	0.000	0.954-0.981
History of Diabetes Mellitus	1.272	0.0187	0.000	0.614-0.855
History of treatment for hypertension	1.020	0.086	0.815	0.864-1.203
History of CVA	0.572	0.075	0.000	0.442-0.741

5.4 Discussion

The prevalence of RPD in the over 65 year Bridlington population is estimated to be 5.06% for either eye, being the highest population based prevalence to be reported to date, being significantly higher than the 0.7% baseline prevalence (in subjects aged 43-86 years) and 3% 15-year cumulative incidence reported by Klein et al in the BDES. It is also considerably higher than the 0.41% prevalence in The Melbourne Collaborative Cohort Study (MCCS), which had participants aged 48-86 years [58]. The difference may be explained in part by the older age-group of our population, and the utilisation and analysis of the red-free channel of the colour fundus photograph, which enhanced RPD detection within this study. To the best of the author's knowledge, no other population based study has previously used red-free enhancement of FP images for RPD detection. Detection of RPD and differentiation from conventional drusen is highly dependent on recognition of the particular fundus features and involves a degree of pattern recognition. There is a likely high inter-observer variability, with the potential for significant under-reporting amongst inexperienced graders and ophthalmologists not familiar with the retinal features of RPD. In this study, the primary grader was an experienced ophthalmologist, with extensive experience in the imaging features of RPD using FP and multimodal modalities. This, and the improved awareness and recognition of this distinctive phenotype, may have contributed to enhanced detection and higher recorded

prevalence. All population studies to date, however, may likely have underestimated the true prevalence of RPD given the utilisation of only FP and its known reduced sensitivity in detecting RPD when compared to other imaging modalities. There remains no gold standard diagnostic test for RPD, but studies have shown that several imaging modalities have greater sensitivity for their detection, when compared to FP. Smith et al in 2009 [178] and [201] in 2013 compared multiple imaging methods for diagnosing RPD. Both studies demonstrated IR imaging to be the most sensitive non-invasive modality (approximately 95%). FP has been shown by Smith et al to have a sensitivity of 88% [178]. The downside of IR is that it had a low specificity when compared to other tests (92%), possibly because soft drusen have a similar appearance on IR [201]. A major criticism of this study is its sole use of FP, rather than multimodal imaging for RPD detection. Although some other milestone studies have used only FP, the diagnostic ability of this modality is considered unsatisfactory by some secondary to its low sensitivity [200]. However, its specificity is extremely high (almost 100%) and therefore it is a powerful confirmatory test [200]. It has been suggested as there is no diagnostic gold standard, at least two imaging modalities should be used [202]. It has been suggested that initial screenings hould be undertaken with highly sensitive imaging modalities, such as SD-OCT, in combination with a highly specific one, such as FP for confirmation [200]. Future RPD/AMD studies should use a multimodal approach for identification.

RPD are known to increase in prevalence with age [37, 203, 204]. In the BDES the highest prevalence recorded was 2.4% in the 75 to 86-year age group. In the

MCCS, the highest prevalence recorded was 2.78% in woman aged over 80 years [58]. In this study, the prevalence reached significantly higher levels (27.3% in the over 90-years age range). In the BEAP, there were gradable photos available in at least one eye for 751 subjects over 80 years of age, allowing a good estimation of prevalence of RPD in the very oldest individuals within the community. This is comparable to the oldest individual at baseline in the BDES was 86, reflecting the relatively old population in this present study.

Reticular lesions have consistently been reported to be more prevalent in females, with Arnold et al reporting that 87% of RPD identified were in woman [30]. Smith et al reported a similar 79% female preponderance [178]. Klein et al, after controlling for age, reported a 2 ½ fold increase in prevalence and incidence in woman compared to men [37]. In the present study, RPD had a higher gender specific prevalence in women. To date, the disproportionate occurrence of RPD in females has not been adequately explained. One suggestion is that RPD may be of an autoimmune inflammatory origin [37]. This has been proposed as an explanation because of the higher prevalence of such diseases in females, supported, in part, by the association with a history of steroid eye drop use in the BDES [37]. An alternative hypothesis, however, is that as RPD has been associated with a 54% decreased survival rate in subjects at baseline in the BDES, there may be a systemic cause for RPD that confers an increased mortality among males at a younger age. This hypothesis is supported by the increasing evidence of an association between RPD and risk factors for

cardiovascular disease, such as hypertension [57] and angina [205]. A recent publication has demonstrated an association with diffuse-trickling GA with cardiovascular disease, particularly in males [61]. The Fleckenstein group demonstrated that in the under 65-years age-group, 54% of patients had previously been admitted to hospital due to cardiovascular disease, including hypertensive crisis, angina and myocardial infarction. This current study adds further weight to the association with cardiovascular risk factors, given that the strongest association with RPD was a history of DM and a suggestion of a protective effect of hypertension treatment. Boddu et al [57] reports patients with RPD are more likely to be hypertensive than those with large soft drusen. In the MCCS, Finger et al found that RPD were associated with moderately elevated systolic blood pressure, current smoking and a trend for an association with a history of myocardial infarction or stroke [58]. This effect was reported to disappear with multivariate analysis, indicating a modest effect size. In the BDES, while controlling for age, RPD were associated with, lower income (OR per lower income group 1.75, 95% CI 1.16, 2.62, p=0.007), body Mass index (OR per 1kg/m2 263 1.08, 95% 264 CI 1.02, 1.15, p=0.006) and more pack years smoked (OR for 35 or more pack years smoked vs none = 2.61, 95% CI 1.17, 5.85, p=0.02) [37]. In the BMES, Tan et al found that high-density lipopreoteins (HDL) were inversely related to incidence of late AMD [59]. A history of any cardiovascular disease, including stroke, myocardial infarction or angina was also associated with incident early AMD and incident soft or reticular drusen [59]. To the best of our knowledge, the protective effect of hypertension treatment

in relation to RPD is newly reported and clearly warrants further investigation. Additional studies and further analyses are needed to support this finding and assess the impact of different classes of antihypertensive medication, duration of medication use, and establish the influence of blood pressure reduction on risk of RPD development and progression to AMD. Alternatively, this finding could be by chance, given the small sample size and number of individuals with RPD.

To the best of the author's knowledge, a new and previously unreported finding from this study is that RPD appear to be associated with visual dissatisfaction, when adjusted for age. This is not surprising given the known association of RPD with advanced AMD. However, it has previously been demonstrated that RPD decrease the retinal sensitivity within the macular area irrespective of visual acuity level [206]. With microperimetry, Querques et al found a difference in the overall function of the macula between patients with RPD and those with only soft drusen. In this present study, there appears to be a suggestion of a tendency towards visual dissatisfaction in subjects with RPD. This may be explained by photoreceptor dysfunction in the presence of RPD. Alternatively, eyes with RPD and the associated choroidal thinning may form part of a spectrum of chorioretinal changes seen in age-related choroidal atrophy (ACA), which is known to be associated with mild reduction in vision [207]. The retinal phenotype described by Spaide, including a tessellated fundus, peripapillary atrophic changes, rarefaction of choroidal vessels in the macula and central

pigmentary changes were sometimes identified in eyes with RPD. However, given the often subjective nature of grading such changes on FP, especially in the absence of enhanced depth imaging to allow choroidal thickness measurement, no association has been objectively explored.

In the first population based report of RPD prevalence, Klein et al [37] mapped the topographical distribution of RPD within the ETDRS grid. The authors reported a zero rate of involvement of the central subfield, using FP as the imaging modality. Foveal sparring is now a well-recognised phenomenon of RPD [182-184]. However, several studies utilising multimodal imaging techniques have reported central subfield involvement, albeit at a lower rate. Using confocal SLO, Steinberg reported central subfield involvement in 46%, which was significantly lower than the rate for the surrounding subfields (62-100%)[183]. A study by Smith et al, using IR has similarly demonstrated RPD involvement within the central macula. They suggested that the blue light used in other modalities is suppressed by luteal pigments, accounting for the apparent absence of reticular lesions in the central zone [178]. In this present study with colour FP central subfield involvement is significantly lower than all of the surrounding subfields, adding further support to the notion of relative foveal sparring from RPD. This study further confirms the predilection of RPD for the superior macula. The author acknowledges that this measure of foveal involvement, along with adjacent subfields, is likely an underestimation given the use of FP alone. Multimodal imaging, particularly SD-OCT, which would have allowed the identification of SDD, would have been ideal in helping to differentiate small

central RPD from conventional hard drusen with confidence. In eyes with comorbid drusen or pigmentary change, grading with confidence using FP alone is not possible, resulting in a potential underestimation. It is recognised that RPD involving the central subfield (on FP) may have a particularly distinct appearance that has been referred to as 'ultrafine RPD', by the author (above)that may form part of a continuum of the central stippled pigment epitheliopathy often seen in association with RPD in a foveal sparring GA.

To the best of the author's knowledge, to date there is no reported breakdown of RPD prevalence into subtypes based on their fundus features from within a community based population study. Limited literature is available on the subject, even in hospital based populations [198, 199]. This is in part explained by the lack of consensus regarding RPD sub-classification. Currently there is no recognised diagnostic gold standard test, nor is there a definite internationally accepted description for the utilisation of multimodal imaging and subsequent sub-classification. Suzuki et al [199] reported dot RPD as the most commonly identified subtype, found in 96.1% of all evaluated eyes. This present population based study confirms that dot RPD is a frequent finding, identified in 54.8% of eyes with RPD. The lower prevalence in the present study may, however, reflect the different study population (community based cross sectional study in the BEAP) versus a hospital based retrospective review of patients with known RPD [199], where the RPD may be more obvious or more advanced resulting in referral to a hospital eye service. An alternative explanation is the different

diagnostic imaging tools utilised in the different studies. In the BEAP, only FP with digital enhancement was used, while both FP and IR were used in the study by Suzuki et al [199]. They specifically reported that dot RPD were most commonly identified using IR, and whilst ribbon RPD was more detectable using FP. As the present study used FP, ribbon RPD were the most frequently identified subtype, in agreement with the Suzuki study. This study, further demonstrates that the different subtypes of RPD can occur together or independent of each other. It is, therefore, reasonable to assume that one type is not a more advanced form of RPD development. It remains to be determined if dot or ribbon RPD confer different risks for the development of either GA or nAMD.

In agreement with several previous reports, this study confirms that RPD are highly associated with advanced AMD, with an overall prevalence in subjects with either GA, nAMD or, most likely, a PPCVM in their worse eye of 25.9%. Smith et al reported that 74% of subjects with RPD had late AMD, with nAMD being the predominant subtype [178]. In the MCCS, Finger et al report GA to have the strongest association with RPD [58]. The reasons behind these differences may simply reflect the tendency for RPD to regress with incident GA or the development of CNV. Given the predilection for RPD within the supero-temporal arcade, it is likely that RPD would be more easily identified in eyes with a PPCNV, as the macular area is frequently preserved. In this study we report that only a minority of eyes (13%) with RPD have no or minimal signs of ARM, highlighting the high concurrence of RPD other with other retinal features of AMD.

In conclusion, this study demonstrates that RPD are likely to be a more common finding than previously reported in population based studies, with a prevalence with digitally enhanced FP of approximate 5% in the over 65 population. The prevalence increases significantly with age, reaching a maximum of 27% in the over 90 year age group. This study confirms that RPD most frequently occur in the upper outer macula subfield, and that they do occur within the central macula subfield contrary to previous reports. RPD occur with a female preponderance. They are commonly found in association with other signs of ARM, including drusen over 125µm (50 % of the time) and pigmentary changes. Isolated RPD, in the absence of conventional drusen, is uncommon but does occur. Approximately 1 in 4 subjects with advanced AMD will have evidence of RPD in either eye. There is a suggestion that RPD are associated with visual dissatisfaction. However, this will require further exploration.

Figure 5: RPD associated with FP features that can be suggestive of ACA.

There is a tessellated fundus appearance secondary to patchy alteration in the density of choroidal pigment, a rarefaction of choroidal vessels nasally with enhanced scleral show and clumping of RPE pigment.

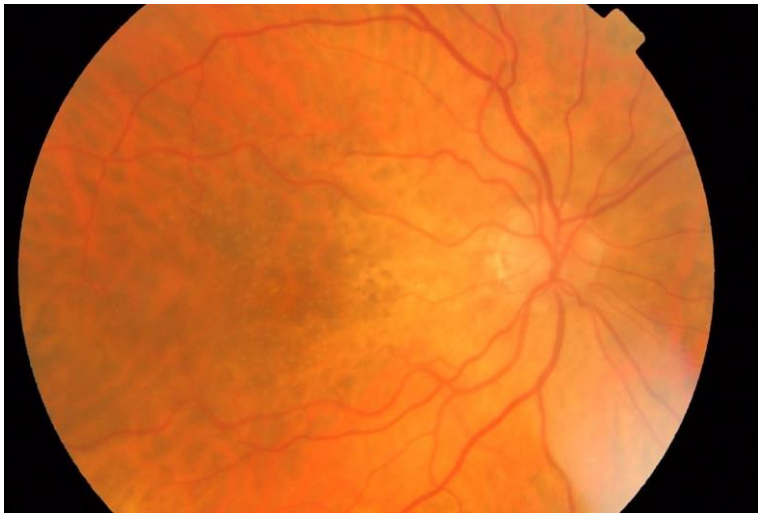


Figure 6: RPD with central subfield involvement.

Very small RPD can pose difficulties with grading using FP alone.

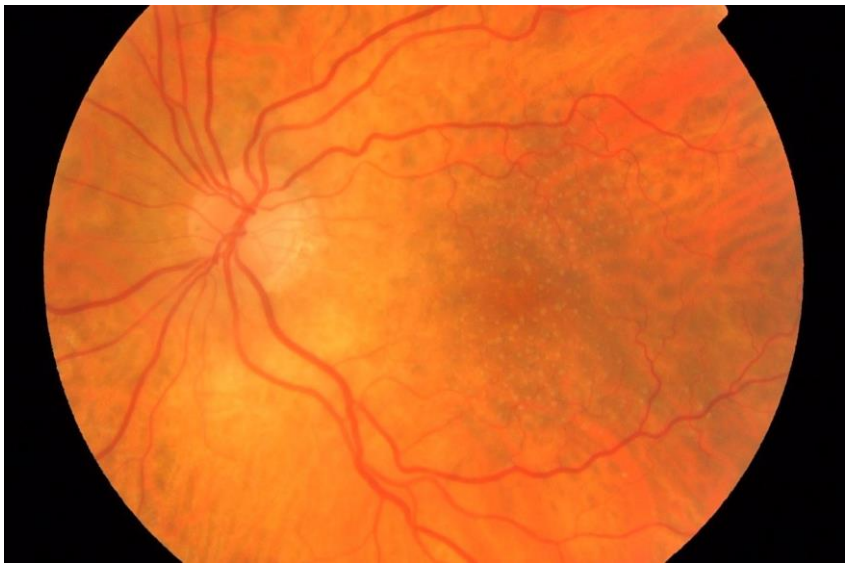


Figure 7: U shaped foveal sparring GA, with clearly visible superotemporal RPD.

In the preserved central region there are what appears to be regularly arranged and densely arranged 'ultrafine' RPD, associated with RPE pigmentary changes.

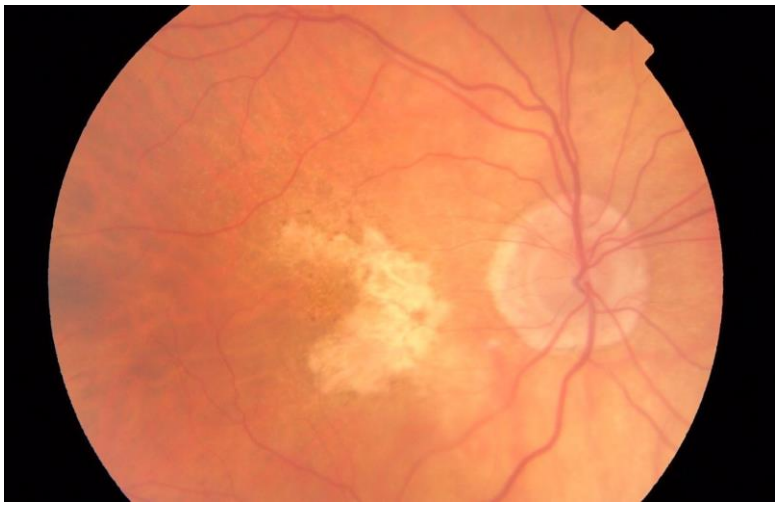


Figure 8: Topographical distribution of prevalent RPD for the right eye in the BEAP.

Number of eyes =140. Rates for each location are not mutually exclusive.

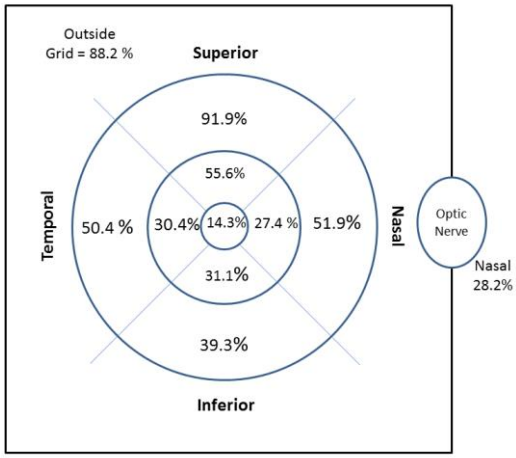
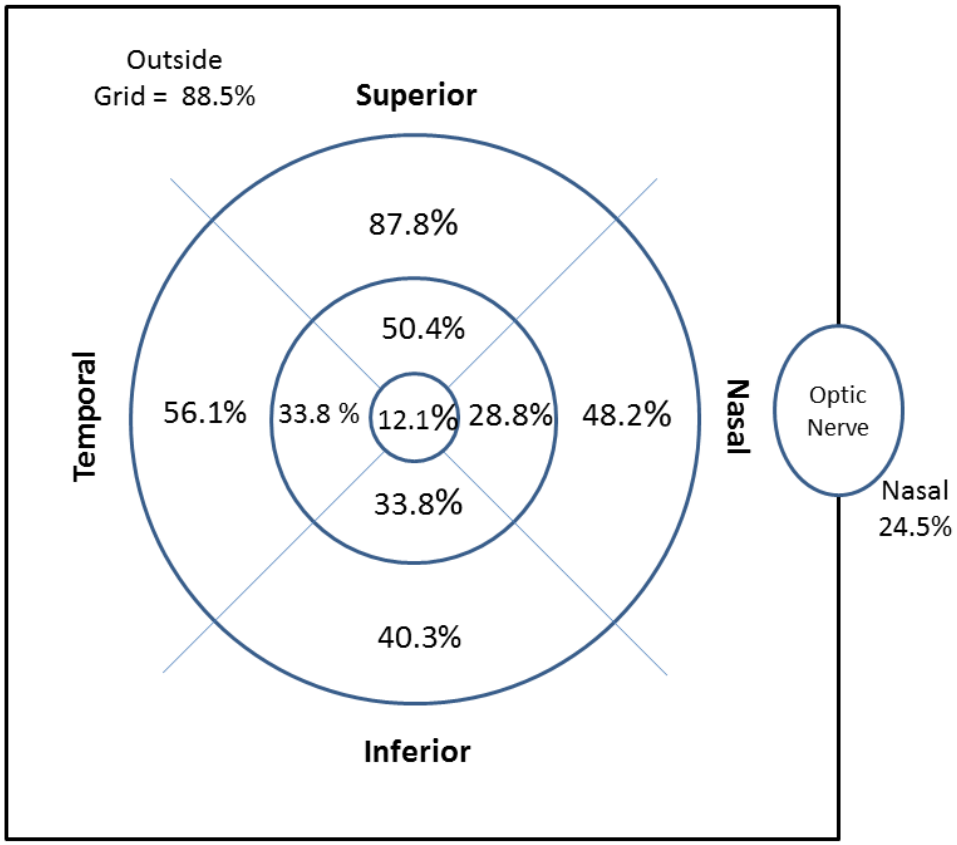


Figure 9: Topographical distribution of prevalent RPD for the left eye in the BEAP.

Number of eyes=141. Rates for each location are not mutually exclusive.



Chapter 6: Prevalence of peripapillary choroidal neovascular membranes (PPCNV) in an elderly UK population

6.1 Introduction

PPCNV are an important condition, and form part of the pathological spectrum of a number of diseases. They have the potential to cause severe loss of vision [208]. They are well-recognized but uncommon, accounting for less than 10% of all newly presenting CNV [209, 210]. In a survey by Browning and Fraser, [211] PPCNVs were reported to be associated with AMD in 45% of cases while 39% were recorded as idiopathic. PPCNVs have been associated with several other conditions including inflammatory diseases, such as presumed ocular histoplasmosis [212], uveitis [213-216], and chorioretinitis [217]. Degenerative processes including myopia [218] and angioid streaks [211] are also reported associations. Choroidal osteoma, optic disc drusen and congenital disc anomalies are other rare associations. Our team has previously reported that PPCNV occurred in 9 out of 231 cases of AMD in a hospital setting (3.9%) [209]. Most or all of these previous reports and associations are based on case reports or small case series, and based on data from hospitals. To the best of the author's knowledge, there are no reports on PPCNV derived from a community setting. As such, there is no data on the population prevalence of the predominantly asymptomatic PPCNV, except for that in chapter 3, in which we reported a worse eye prevalence of 0.3% compared to 1.8% for nAMD (grade 4b AMD) and 2.5% for GA (grade 4a AMD).

In this study, the characteristics of eyes classified as having PPCNV (grade 4c AMD) from the BEAP Study were investigated. The study provides invaluable information on the characteristics of asymptomatic PPCNV, which until now remains unreported.

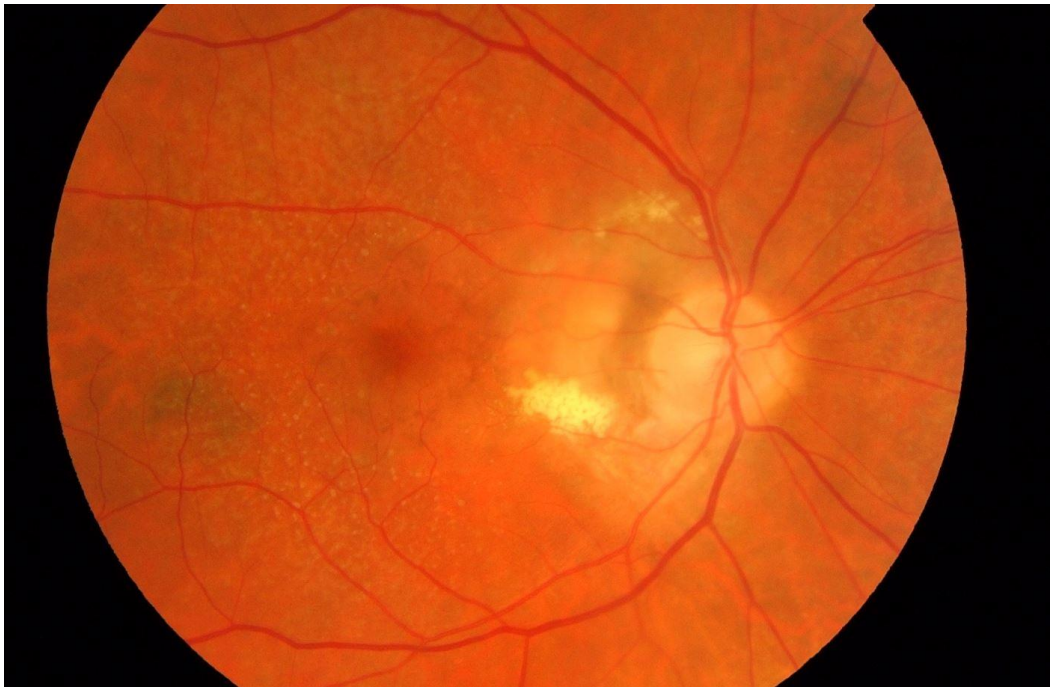
6.2 Methods

The methods of BEAP, image acquisition and analysis are discussed in detail in the Methods of Chapter 2 and 3. All eyes identified as having PPCNV were subsequently reviewed in greater detail. For each eye with a PPCNV, the signs of ARM within the ETDRS macular grid were also recorded using a modified Rotterdam grade to assess age related changes as discussed in Chapter 3. RPE changes and the presence of drusen in the peripapillary area (one-disc diameter around the optic disc) were also specifically recorded. The outline of the PPCNV was drawn and the area of involved retina measured. The PPCNV area was taken to include only visible membrane (as indicated by a grayish-brown or whitish-grey appearance) or areas of sub-retinal or sub-RPE haemorrhage. Areas of obvious peripapillary glial tissue, if associated with haemorrhage were also measured as part of the lesion. Often, the membrane would be located adjacent to the disc, with peripheral haemorrhage or exudate. Areas of SRF without any haemorrhages were not in themselves measured as part of the lesion. Only gross areas of exudation immediately adjacent to haemorrhage or a membrane were included in the area measured, whereas sparse distal exudates were not. The extent/severity of the PPCNV was recorded using clock hours of

involvement of the circumference of the optic disc. The location of the PPCNV was also recorded. An example of a PPCNV is shown in Figure 10.

Details of the macular changes of ARM/AMD in the contralateral eyes were also recorded, along with the closest distance from the edge of changes secondary to the PPCNV, including SRF to the fovea.

Figure 10: An example of a PPCNV, with visible membrane and exudation. There are visible RPD.



6.3 Results

Amongst the total of 3475 participants with gradable photographs in at least one eye, PPCNV were identified in a total of 10 subjects (summarized in Table 20. PPCNV were a bilateral finding in 2 subjects (20%), resulting in a total of 12 eyes with PPCNV. Their ages ranged from 66-85 years, with a mean of 76.3 years (SD 6.4). Seven individuals were female (70%), with gender specific prevalence rates of 0.36% (7/1939) and 0.19% (3/1536) for females and males respectively. Bilateral involvement was very rare with a community prevalence of 0.06%. There does not appear to be an increase in prevalence with age, as seen with both nAMD and GA as shown in Table 21 (p=0.77), the number of cases is, however small. Only one subject had identifiable angioid streaks. No other individuals with PPCNV had pathological myopia or optic disc pathology such as disc drusen or disc swelling, or evidence of previous chorioretinitis. Visual function was good in the identified subjects at the time, with no individual having poor VA secondary to a PPCNV. Eyes with reduced vision often had co-morbid conditions. No individual was felt to have direct involvement of their fovea with SRF, exudate or haemorrhage at the time of fundus photography. Patient Number 10 (VA 0.22) had a co-morbid epiretinal membrane. Patient 2 had presenting LogMAR VA of 0.42, but subsequently underwent cataract surgery and postoperatively had a VA of 0.2. Subject 4 had a VA of 0.3 at presentation. This patient was reviewed in clinic for 23 months

with no documented progression. They were reported to have a haemorrhagic peripapillary scar with atrophy and discharged. Their VA reduction may have been in part secondary to macular RPE changes.

Three subjects (30%) with PPCNV had RPD. Nine (9) out of 10 individuals (90%) had evidence of drusen ≥63µm in size within the macula area, including the one patient who had angioid streaks. One individual (10%) had contralateral nAMD and another subject had bilateral GA that appeared completely separate from any PPCNV (which was nasal) with multiple large drusenoid pigment epithelial detachments, some of which appeared to be regressing. All individuals had RPE hyperpigmentary, hypopigmentary or atrophic changes around the optic disc. Table 22 details the demographic and macular changes in the individual participants with PPCVN. Table 23 summarises the peripapillary retinal changes along with size (area) and locations of CNV in relation to the optic disc in all the eyes with PPCNV. One eye (8.33%) in this series had a large PPCNV involving 6 or more clock hours. Six (6) of the 12 identified PPCNV had a predominantly temporal location with another occurring in a superior position. Five of 12 PPCNVs identified (41.7%) involved only the retina nasal to the optic disc. The PPCNV size ranged from 0.46 $mm^2$to 7.93 mm^2 with a mean of 2.81mm^2 (SD 2.82). The mean area of PPCNV in eyes with visible exudation was 5.64mm^2 compared to eyes with no exudation, where the membranes measured an average of 0.9mm^2.

Table 20: Population prevalence of PPCNV. Data is number (%) [95%CI]

Grade	Right Eye (3340 gradable photos)	Left Eye (3384 gradable photos)	Participants (3475 gradable photos)	Bilateral (3255 participants with gradable photos in both)
4c	6 (0.18) [0.07-0.40]	6 (0.18) [0.07-0.40]	10 (0.29) [0.15-0.54]	2 (0.06) [0.01-0.24
Mean Age (years)	76.7	73.6	76.3	69.5

Table 21: Age specific prevalence of PPCNV. Data is number (%). [95% CI]

AMD Grade	65-69	70-74	75-79	80-84	85-89	>90	Total
4c	2 (0.24) [0.01-0.91]	1 (0.09) [0.01-0.58]	3 (0.37) [0.07-1.14]	3 (0.56) [0.11-1.72]	1 (0.55) [0.01-3.34]	0 (0.00) [0.00-12.39]	10 (0.29) [0.15-0.54]
Total	849	1069	808	533	183	33	3475

Table 22: Summary of demographic details of eyes with PPCNV and associated macular age-related changes (Rotterdam Grades), with visual acuity (VA)

	Participant study number	Affected eye	Age at exam	Sex	AMD Grade Right	AMD Grade Left	Right VA	Left VA	Cause of Vision loss
1	1328	LE	66	F	1a	1a	0.02	0.14	nil
2	1710	LE	76	F	1a	1a	0.30	0.42	Other: cataracts
3	2127	LE	76	F	1a	1a	0.1	0.1	nil
4	133	LE	85	M	4b	4, reticular drusen	CF	0.3	ARM
5	2708	RE	83	F	0a	0b	0.2	0.2	nil
6	1150	BE	72	F	1a	1a	0.1	0.1	nil
7	2685	RE	77	M	2a, reticular drusen	2a, reticular drusen	0.2	0.2	nil
8	635	RE	80	M	3, reticular drusen	3, reticular drusen	0.0	0.0	Nil
9	741	BE	67	F	4a	4a	0.3	0.4	RE AMD-GA, LE diabetic CSMO
10	1860 (angioid streaks)	RE	81	F	1a	Cannot grade (Non-AMD)	0.22	0.36	RE-ERM, LE-cataract

Table 23: Summary of the peripapillary retinal changes along with size (area) and locations of CNV in relation to the optic disc in all the eyes with PPCNV.

Study number	Affected eye	Features of the peripapillary disc area ipsilateral eye	Features of the peripapillary disc area contralateral eye	Maximum drusen diameter (μm) ipsilateral eye	Maximum drusen diameter (μm) contralateral eye	Signs of PPCNV	Area of PPCNV (mm^2)	Distance from edge of lesion to fovea (mm)	Clock hours
1328	LE	RPE atrophy	RPE hyperpigmentary crescent and some RPE atrophy	90	210	RPE changes and subretinal haemorrhage, visible membrane	3.90	2.89	3 (11-2 S)
1710	LE	Alpha zone changes with hypopigmentation and drusen visible	A pigmented crescent of RPE hyperpigmentation temporally with more widespread hypopigmentation	250	130	Peripapillary haemorrhage and RPE changes	1.39	5.68	2.5 (6.30-9 IN)
2127	LE	Hypopigmentation around disc with drusen	Focal area of RPE atrophy with scleral show. RPE hyperpigmentation	250	160	Multiple peripapillary subretinal haemorrhages and exudate	6.49	2.18	5 (12.30-5.30 T)
133	LE	There is a rim of RPE atrophy around the disc with extensive atrophy beyond	Extensive atrophy	150	0 (4b)	Haemorrhage surrounded by disciform scar and atrophy	1.23	2.21	1.5 (2.30-4 T)
2708	RE	There is a RPE hyperpigmentary crescent and areas of hypopigmentation and	Small area of RPE atrophy with more widespread hyperpigmentation/hypopigmentation	0	≤63	Subretinal haemorrhage	1.42	4.87	2 (4-6 IN)

		atrophy							
1150	BE	RPE atrophy with hyperpigmentary crescent RE	RPE atrophy LE withy hyperpigmentation and hypopigmentation	200 RE 220 LE	NA	RE-multiple haems and atrophy LE-Haemorrhages and atrophy	0.58 RE 0.46 LE	3.20 RE 3.34 LE	RE: 1.5 (7.5-9 IT) LE: 1.5 (3-4.30 ST)
2685	RE	RPE atrophy with more widespread hypopigmentation	RPE atrophy with more widespread hypopigmentation	190	210	Subretinal haemorrhage	0.63	5.07	2 (12-2 SN)
635	RE	RPE atrophy and reticular drusen	RPE atrophy and reticular drusen	170	190	Gross exudation, visible membrane, retinal thickening	7.93	1.52	6 (12-6 T)
741	BE	RPE atrophy and hyperpigmentation with drusen RE	RPE atrophy and hyperpigmentation and hypopigmentation	450	230	RE: Gross exudation, haemorrhage and retinal thickening LE: subretinal haemorrhage and pigmentary changes	RE: 7.21 LE: 1.49	RE: 6.29 LE: 3.19	RE: 5 (12-5 N) LE: 1 (1-2 ST)
1860	RE	Angioid streaks with RPE atrophy and hyperpigmentation	Angioid streaks	265	Cannot grade as poor quality photo, but questionable PPCNV with haemorrhage	Haemorrhage with exudate	0.94	5.59	1 (4.5-5.5 IN)

6.4 Discussion

This chapter reports the prevalence of PPCNV within an elderly Caucasian population in the UK, using data from the BEAP, the largest population based screening study of AMD to date for an over 65-year population. To the best of the author's knowledge, this represents the first population study that has specifically reported the prevalence of PPCNV, as all previous publications on the subject have arisen from hospital populations, and in predominantly symptomatic individuals, which carries inherent selection bias.

PPCNVs (grade 4c AMD) were an infrequent finding, with a prevalence of 0.29% for individuals over 65-years of age. This is considerable lower than nAMD, which within the same population had a prevalence of 1.8% for the worse eye. In keeping with previous published studies [219, 220] there was a female preponderance, with 70% of PPCNV occurring in females. This difference was maintained in gender specific prevalence rates of 0.36% and 0.19% for females and males respectively. In the natural history study published by Silvestri et al, 70% of patients (over the age of 40 years) were also female [220]. Interestingly, drusen and RPE changes are no more prevalent in women than men. The only retinal degenerative changes known to have such a gender predilection, to date, are RPD. However, it seems unlikely that the increased female prevalence in this study can be attributed to their presence, as the three patients with RPD were both male. We have previously reported PPCNV account for 3.9% of newly diagnosed cases of CNVM's that present to a hospital eye service [209]; other

publications have reported that they account for less than 10% of all CNVMs [221]. This is confirmed in the BEAP, in which PPCNV accounted for 12 out of a total of 90 cases of CNVMs, representing 13.3% of all prevalent membranes identified. Importantly, crude inference that could be made from this is that approximately two thirds of PPCNV will likely remain asymptomatic, a finding that is not surprising when 40% of membranes are located nasally, and the high mortality rate within such an elderly population.

The majority of cases of PPCNV were unilateral, a finding which appears similar to previously published reports. In a hospital population, Silvestri et al reported that for individuals over 45 years of age, 54% of PPCNVs were bilateral. This bilaterality increased with age to reach 62% in the over 70 years age group [220]. However, only 2 out of the 14 (14%) individuals within the series had bilateral disease at presentation, with the remainder developing contralateral eye involvement over a prolonged period of follow up, which in one individual as long as 7 years following presentation. Similarly, Browning et al in their series reported bilateral involvement in 19 of their 96 patients (19.8%) [211]; but that study again involved a median follow up period of 2 years. The present study did not have follow-up data on our participants and cannot therefore comment on the risks of developing similar disease in the contralateral eye.

There is a myriad of published associations between PPCNV and other conditions. These are mainly single case reports or small case series. The larger hospital based studies may give a poor representation of the true community associations between PPCNV and other conditions as small, nasal or age-related

membranes may remain asymptomatic. In a series of 115 eyes of 96 patients, Browning et al reported the prevalence of ocular conditions associated with PPCNV to be: 45.2% ARM, 39.1% idiopathic, 4.3% multifocal choroiditis, 2.6% angioid streaks, 1.7% presumed ocular histoplasmosis, 1.7% choroidal osteoma, 0.9% optic disc drusen and 0.9% congenital disc anomalies. Within their study the definition of ARM was broad. It included all individuals who had 1≥ of the following in both eyes: drusen larger than 63µm, pigment clumps, mottled pigment epithelial atrophy, GA or signs of an exudative AMD including disciform scars. They reported that 39% of subjects with PPCNV had drusen on [211]. Kies and Bird similarly reported that only 15 of their 55 eyes (27%) with PPCNV had identifiable drusen [219]. Silvestri et al reported that 60% of membranes in her series were related to age-related degenerative changes. In 90% of eyes in the present series there was evidence of drusen ≥63µm within the macular area, which is far higher than previously published. The eyes with PPCNV in this study have a higher prevalence of the more the advanced stages of AMD when compared to the overall BEAP cohort. Within the over 65-year population in the BEAP Study, 38% of individuals have no or minimal signs of ARM evident in their worse eye (Rotterdam grade 0), as discussed in Chapter 3. Out of the ten individuals with a PPCNV, only 1 subject (10%) had no or minimal morphological signs of AMD present (Rotterdam grade 0). Overall, 18% of the over 65 year population in the BEAP study had either a Rotterdam grade 2, 3 or 4a in their worse eye. In eyes with a PPCNV, the prevalence of Rotterdam grade 2, 3 or 4a within the macula was considerably higher at 42%. This could be a chance

finding, but it appears to suggest a stronger association of PPCNV with signs of ARM than previously published, at least for individuals over 65 years of age. It is acknowledged that it is possible that some of the pigmentary changes within the macular area could have occurred secondary to SRF, but the FP or the visual acuities were not in keeping with this suggestion. The lower prevalence or ARM reported within the hospital populations could in part be explained by the fact that symptomatic PPCNV (as seen in hospital based cohorts) will more likely be large membranes, and have macula involvement from exudation or SRF. The presence of such changes can make drusen difficult to see or can even lead to drusen regression. Alternatively, it could reflect the older age of the cohort included in the present study. Furthermore, in the current series, 30% of PPCNV were associated with RPD, a finding which has previously been unreported. Although this is significantly lower than the prevalence of conventional drusen, their prevalence among the general population over 65 years is lower, a finding that may be considered significant [37]. RPD cover a large area of the retina and can encroach towards the disc margin. They are already known to be associated with nAMD [179, 222]. In the AREDS their presence was associated with a 5 year incidence of nAMD of 29%. In the BDES their presence at baseline was associated with the highest 15-year cumulative incidence of nAMD (20% as compared to 10% for a soft indistinct drusen) [37]. We have previously demonstrated they have a prevalence of 22% in eyes with newly presenting nAMD [179], which is similar to that of the prevalence in participants with PPCNV in this population.

The finding that 100% of our subjects had RPE pigmentary changes within half a disc diameter of the disc margin (in both affected and contralateral eyes) is an important one. Previous studies have not reported the presence of drusen or pigmentary change within the immediate peripapillary disc area. As drusen and pigmentary changes within the macula are the known hallmarks of both GA and CNV, it seems logical to consider these changes in the peripapillary area as potentially pathological for PPCNV and for them not to be overlooked. It is possible that in some of the previous reports of idiopathic PPCNV (with no macular age related changes evident) the RPE in the area around the optic disc may have demonstrated some age-related degeneration. Other research groups have speculated on the aetiology of this relatively large cohort of presumed idiopathic PPCNVs, and some have hypothesized a relationship with previous unwitnessed episodes of multiple white dot syndromes [223, 224]. A more plausible explanation is that the localized and age-related changes that occur in the peripapillary area, which involve the RPE-Bruch's membrane-photoreceptor complex, can predispose to localized breaks in Bruch's membrane that allow CNV membranes to traverse. Peripapillary degenerative changes in the present series were a more universal finding when compared to the presence of drusen. It is likely that eyes with a 'scleral show' or atrophy of the RPE in the peripapillary area predispose to localized breaks in Bruch's membrane or the proliferation around the termination of Bruch's membrane.

In 10% of subjects with PPCNV in this study, there were identifiable angioid streaks. This individual was removed from the initial report of AMD prevalence within the UK (chapter 3) as the pathology was not felt to be purely age-related. The patient was 81 years of age and did have co-morbid large soft drusen within the macular area. The association with angioid streaks and PPCNV is well established [225-228]. This study reports a more frequent association than previously published (although the numbers are small), and highlights an overlap between the aetiologies of PPCNV in patients with angioid streaks. For comparison, Browning et al reported 2.6% of PPCNV were associated with angioid streaks [211] and Silvestri reported no cases associated with angioid streaks in her series. This could be a reflection of the small numbers involved, or reflect the fact that perhaps membranes that occur in the region of an angioid streak remain small and asymptomatic and, therefore, are less likely to present to a hospital eye service. In the case reported here, the membrane was small and the individual eye maintained good vision. Perhaps the increased prevalence in this present series reflects the increased risk for the development of a PPCNV in individuals with angioid streaks as they age.

The extent of the PPCNV in this series was quantified using clock hours of disc involvement. Some authors have defined a PPCNV as being large if it covered more than 3.5 disc areas or involved over 50% of the disc circumference[229]. Only one subject (10%) in the current series had 6 or more clock hours involved, with this participant having co-morbid RPD. This figure is very similar to the 15% prevalence of large membranes reported by Browning et al [211] in their series of

115 eyes. Kies and Bird [219] reported a much higher prevalence of large membranes involving more than 6 clock hours (87%) in their Moorfield's cohort. This, however, as suggested previously by others [211], may reflect a selection bias with extremely severe cases being referred onwards to this large international center. Kies and Bird [219] also reported complete disc encirclement occurred in 6 of 55 eyes (11%), while Browning et al reported a lower prevalence of complete disc encirclement (0.9%) [211]. Caution has to be exercised when comparing area of CNV given the different imaging modalities used. One significant limitation of the present study includes the use of non-stereoscopic FP (field 2), centered on the macula. Other studies in hospital settings have used FFA to quantify the extent of the PPCNV, and this will inevitably result in a better delineation of the PPCNV. Approximately half of PPCNV on FFA are entirely or mainly occult lesions, often with poorly defined borders, which can make the precise location of the membranes difficult to ascertain, even with the use of FFA [211, 219, 230, 231]. It has been reported that subretinal neovascular complexes removed at subretinal surgery have been documented to be larger than predicted from the FFA [232]. Kies and Bird [219] also reported that half the cases of recurrence following treatment with laser occurred from an area outside the treated region: areas in which there was no identifiable membrane visible with the pretreatment FFA. This finding may indicate that the PPCNV are larger than delineated even with the use of FFA. For these reasons, some authors previously (when treatment with laser ablation was more common) advocated for the use of indocyanine green angiography to more adequately delineate the

margins of occult lesions prior to any considered laser treatment [230]. It is, therefore, acknowledged that accurate PPCNV localization and measurement with FP will have inherent shortcomings.

Untreated PPCNV are known to have a varied clinical course, ranging from spontaneous involution, stabilization or extension towards and involvement of the fovea [233]. Their behavior seems heterogeneous, with rates of progression that appear unpredictable [216, 220, 233, 234]. Some authors have suggested differing visual prognosis for PPCNVs of different aetiologies. Browning reported that their data suggested that ARM-associated PPCNVs presented with worse median VA (20/40), and ended with worse median VA (20/70) when compared to idiopathic membranes (20/30 vs 20/32) over a median follow-up period of 2 years [211]. Silvestri et al [220], however, reported a poor visual outcome in individuals over 40 years. In all but one of their reported cases (in the older individuals), there was direct progression of the membrane or deposition of lipid exudate in the subfoveal area. In all such cases, VA was reduced to 6/60 or worse[220]. One individual who was 74 years, reportedly maintained a good VA, but the membrane was documented to be enlarging. In that series, younger individuals, who had unilateral disease of identifiable aetiology, such as chorioretinitis, generally had more favorable visual prognosis. Silvestri et al highlighted the importance of the cause of reduced VA. Individuals with visual loss secondary to peripapillary haemorrhage or serous detachment of the macula generally had a more favorable prognosis. However, those that had direct subfoveal membrane extension or lipid exudation into the foveal region,

generally had a poor visual prognosis[220]. This supports the earlier assertion that the patients in hospital based studies will have significant selection bias towards more severe and more progressive membranes that have already resulted in visual loss and presentation to a hospital eye service. In the present series, the mean area of PPCNV in eyes with visible exudation was 5.64mm^2 compared to eyes with no exudation, where the membranes were on average considerably smaller (0.9mm^2). This finding supports the aforementioned association with poor VA and exudates [220], and likely reflects their increased size and activity.

No individual in this series was thought to have developed direct visual loss from PPCNV. However, it cannot be ascertained whether some of the patients had previously developed SRF that caused the macular changes seen. It is also acknowledged that the measured prevalence may be an underestimation. Bird and Kies have previously reported that in their series of symptomatic PPCNV, 93% were occult lesions, with ill-defined margins on FFA. Often the PPCNV would evolve and lose their capillary content, allowing the retinal oedema and SRF to resolve, and to leave only atrophic choroid or scar tissue [219]. There would sometimes be a progression of the CNVM towards the fovea, with a trail of RPE atrophy in the PPA as evidence of the pathological origin of the membrane. They further reported the finding of an occasional feeder vessel passing from the peripapillary choroid to the CNV at the fovea [219]. It cannot be ruled out that some of the patients in the BEAP study may have had mixed membranes in this way. Some subjects may have been classified as macular CNVM (Rotterdam

stage 4b), but the CNV could have originated in the peripapillary area. However, such scenario would be uncommon.

In the present study of asymptomatic individuals, we found that a large proportion of PPCNVs (42%) were nasal to the disc margin. The literature search could not find any studies on the prevalence of PPCNV in asymptomatic individuals. Sarks provides the best insight on the subject with a clinicopathological correlative study of 150 eyes of 80 patients obtained post-mortem [235]. Sarks identified CNVMs pathologically in the peripapillary area in 14%, macular area in 20%, and in the peripheral retina in 24.6% of eyes. Unlike the large temporal PPCNVs occurring in the eyes of symptomatic individuals with extension to the fovea, as described by Gass and others [114, 220, 235], Sarks suggested that in older subjects, small and frequently nasal asymptomatic PPCNV occurred with greater frequency than large, temporal membranes with macular involvement. Sarks demonstrated that PPCNVs originate from choroidal vessels passing either through breaks in Bruch's membrane or from vessels extending around the termination of Bruch's membrane. Out of the 21 eyes with PPCNV, 12 (57%) had small tufts of CNVM passing through a defect in Bruch's membrane within one-quarter of the disc diameter from the edge of the disc; with 75% of these breaks occurring on the nasal side of the optic disc. The remaining 9 eyes (43%) failed to demonstrate a break in Bruch's membrane, and the new vessels instead were found to grow around the very edge of the Bruch's membrane. In the majority of these 9 eyes (8 out of 9, 89.9%) the CNVM occurred on the nasal side of the disc [235].

Limitations of this study include only having FP of field 2. In addition FP of field 1, centered on the optic disc would have possibly helped to clearly delineate the extent of nasal membranes. SD-OCT imaging around the optic disc and FFA may have improved case detection. Several eyes were identified with peripapillary haemorrhages and further multimodal imaging would have been required to attribute a definite pathology as aetiological. All individuals who were registered as blind or partially sighted were excluded from study inclusion. We cannot be sure that some of these may have had poor vision secondary to PPCNV either bilaterally or in combination with other visually significant ocular pathology. In this way our prevalence measure will likely be an underestimation.

In summary, it is concluded that PPCNV are an infrequent finding (0.29%) within an elderly community population. There is a suggestion of a clear female preponderance. This study re-emphasizes the heterogeneity of symptoms among patients with PPCNV; with a typical characteristic of being asymptomatic in the population, and differs from those previously published using hospital populations. PPCNV can range from large temporal membranes with gross exudation and the potential for severe visual loss, as reported in several symptomatic hospital populations [219, 220], to smaller asymptomatic membranes as reported here and by others [235]. The fact that the individuals in the current series remained asymptomatic is not surprising, especially when approximately 41% of membranes occurred on the nasal side of the disc, with no evidence of

macula extension. This finding is very distinct from that in some previous hospital studies of symptomatic patients. The findings here support the early histopathological study by Sarks [235] which suggested that in elderly individuals PPCNV frequently occurred in a nasal position and often remains asymptomatic. This study suggests by inference, up to two thirds of PPCVN may remain asymptomatic.

A strong association between PPCNV and signs of ARM within both the macula and immediate peripapillary areas is reported. In addition, this study reports the universal finding of peripapillary degenerative changes in all patients with evidence of PPCNV. RPD were present in 20% of eyes with PPCNVs. There remains a paucity of data on PPCNV in population studies, particularly their association with peripapillary age related degenerative changes. More research is required regarding the natural history of these membranes, including those that are located nasally. More research is also required in order to identify reasons for their apparent heterogeneity in progression rates, utilizing multimodal imaging such as OCT, FFA and ICG in order to help identify possible phenotypic variants.

Chapter 7: The diagnostic accuracy of SD-OCT vs FFA

7.1 Introduction

AMD is the leading cause of visual impairment in elderly patients across developed countries [3] and the third leading cause of global blindness [236]. The prevalence of AMD has been reported across a range of populations and measures as high as 25% for the over 75 year age group [15]. Various classification systems have been used for AMD. They reflect both the population being assessed and ongoing progress with diagnostic imaging and treatment modalities available [27, 43].

Stereoscopic Fundus fluorescein angiography (FFA) is the current gold standard for the diagnosis of nAMD, as currently recommended by the Royal College of Ophthalmologists in the UK [64]. It permits a functional assessment of the retinal and choroidal circulation as well as identification of a breakdown in the blood retinal barrier. The current FFA classification system for nAMD is a historical throwback to the era of laser photocoagulation and photodynamic therapy (PDT) and reflects the need to identify predominantly classic lesions that are eligible for treatment with this now infrequently used management [77, 237]. Despite the widespread use of FFA, the imaging modality does have some significant drawbacks. Firstly it is an invasive procedure, requiring appropriate written consent prior to it being performed. Serious adverse reactions are extremely rare but are known to occur [65]. Yannuzzi et al (1986) estimated the

risk of death following FFA to be 1: 222,000 [66]. Minor adverse reactions are not uncommon. Patients have to be cannulated, which can be difficult in patients with poor venous access. It is essential that facilities for resuscitation be available. FFA is time-consuming, taking around 15 minutes to complete. The quality of the images attained is very operator dependent and requires a good deal of skill and experience: a role generally undertaken by specially trained ophthalmic photographers. If an ophthalmic photographer is unexpectedly ill or away from work for a prolonged period there could be significant delays in diagnostic imaging and initiation of treatment. In some Macular Clinics it is difficult to perform FFAs on the day of presentation for patients with suspected exudative AMD. It often requires forward planning and patients are frequently brought back for separate appointments for a booked FFA. Thus, FFA can be a potential delaying step in the initiation of patients' treatment, as well as adding inconvenience.

OCT was introduced in 1991 and quickly became a widely used imaging technique for a range of ocular diseases that affect the choroid and retina[238]. Unlike FFA which detects the presence of leakage, OCT provides 3-dimensional structural information. OCT is increasingly used to determine both the presence and activity of CNV and the need for treatment/re-treatment in clinical trials and routinely in clinical practice [67, 68]. It is non-invasive, quick to perform and the machines are typically easy to operate, and have a quick learning curve for their use. It has therefore been suggested that OCT imaging may be sufficient in the diagnosis of nAMD, allowing prompt initiation of

therapy. Surprisingly, however, the role of OCT as a diagnostic tool in the setting of a specialist AMD clinic has not been widely investigated. There are very few clinical studies to date that have evaluated the accuracy of OCT imaging against the gold standard of stereoscopic FFA for the diagnosis of nAMD [71-75]. Across the few studies performed a wide range of different sensitivities and specificities for the detection of CNV are reported, in part reflecting the mixed use of either the earlier time-domain (TD) or more contemporary SD-OCT machines and the grading methods used. To the best of the author knowledge, no publication has assessed the diagnostic accuracy of SD- OCT as compared to non-stereoscopic FFA as the reference standard. This may reflect a more real world clinical comparison as many Ophthalmologists do not regularly use a stereoscopic viewer while reviewing fluorescein angiograms in their routine clinical practice.

7.2 Materials and Methods

A retrospective review of all SD-OCT, FP and FFA of 411 consecutive patients (822 eyes) that were referred to a rapid access macular clinic in the Kings' Mill Centre (Kirby-in- Ashfield), over a 4 year period (February 2009 to February 2013) was performed. Inclusion criteria included all patients over 50 years of age that were referred for suspected nAMD by optometrists, general practitioners or other ophthalmologists and had symptoms of reduced vision, metamorphopsia or signs suggestive of nAMD, as determined by the referring clinician. In order to make our results comparable to real world clinical practice

patients that may have had treatment 6 or more months previously with PDT or anti-VEGF but were thought to have new CNV lesions were included in our study as were myopic individuals over 50 years of age. Exclusion criteria included all patients that had either no SD-OCT or FP/FFA available for analysis or those patients where one imaging modality was deemed un-gradable. In addition, if the SD-OCT or FFA were not performed within 7 days of each other the patient was excluded. Patients with CNV secondary to other causes were excluded. No patients were excluded on the basis of their best corrected visual acuity.

FP was performed using the Topcon TRC-50DX, Type IA retinal camera (Topcon, Tokyo, Japan) combined with an attached Nikon D7000, 16.2 Megapixel camera. The operator was an experienced ophthalmic photographer. Bilateral 35° mydriatic non-stereoscopic photographs of field 2 (centered on the fovea) were reviewed. The standard FFA protocol included 35° images of the transit phase, mid phase and late phase up to 10 minutes. Images were all reviewed non-stereoscopically.

SD-OCT images were acquired using the Topcon 3D OCT-1000 instrument (Topcon, Tokyo, Japan). The machine was set to perform a 3D scan with a resolution of 512 by 128 and a scan length of 6.0mm by 6.0mm.

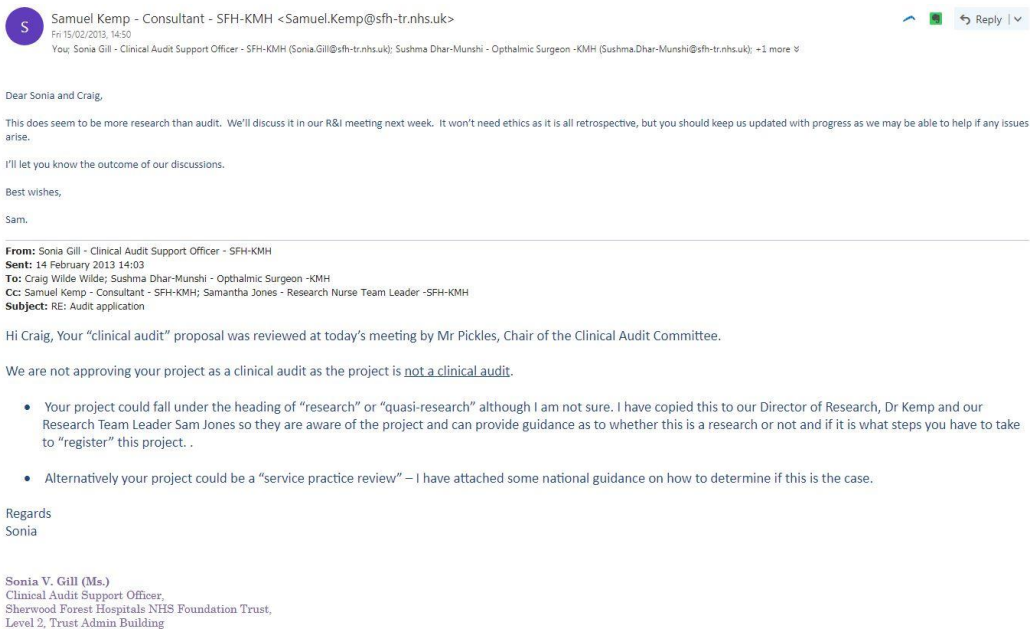
All SD-OCT images were graded by at least two ophthalmologists with appropriate experience in AMD image grading (CW, MP, and AL). SD-OCT images were all reviewed without reference to the FFA or FP. The grader was blind to any clinical patient information such as history, visual acuity or which

eye was the index eye, if not both. Side by side independent grading took place with immediate open discussion and adjudication. If there was disagreement between the two grading ophthalmologists or they were not 90% confident of their assigned grade then adjudication by a third ophthalmologist would take place. After the assignment of the SDOCT grade, the patient's mydriatic FP was reviewed. Repeat grading of the SD-OCT was performed. It was documented if reference to the FP changed the diagnostic grade assigned.

FFAs were graded by at least two ophthalmologists (CW, AL, and WA) independently but side by side, and blind to both the SD-OCT grade and all clinical information. If disagreement existed or the lesion was questionable then adjudication would take place. There was a temporal delay between the grading of all SD-OCT images and its corresponding FFA of at least 4 weeks. For FFA, CNV lesions were graded using the Macular Photocoagulation Study (MPS) grading protocol that was utilised in the treatment of AMD with laser photocoagulation, and photodynamic therapy (TAP) and verteporfin in photodynamic therapy (VIP) studies [77, 237, 239]. Classic CNV was identified as an area of uniform and early (less than 30 seconds) hyperfluorescence that showed leakage throughout the mid and late phases. Occult CNV was identified by areas of increasing stippled hyperfluorescence that appeared in the mid and late phases of the FFA with a leak or a late leak of undetermined origin. FFA images were graded as either: classic, predominantly classic, minimally classic, occult, disciform scar, PPCNV, no CNV or other pathology. CNV was considered present on FFA if classic or occult leakage was detected.

With OCT, CNV was considered present with the grading of changes at the levels of the inner choroid, the RPE or the retina as summarised in below in Figure 11, Figure 12, Figure 13, Figure 14, and Figure 15. Other OCT grades were GA, drusenoid PED, nil CNV, other ocular pathology and disciform scar.

All research complied with the Declarations of Helsinki and all appropriate data protection and ethical standards were upheld throughout the project. The director of research at KMH (Dr Kemp) and research team leader (Sam Jones) were informed of the project and sent copies of the protocol prior to starting the study. The appropriate representative of the ethics committee stated that formal ethics approval was not required as the project was retrospective, non-interventional and involved only the review of images, and not involving direct patient care. A screenshot of e-mail correspondence showing this is inserted below:



Samuel Kemp - Consultant - SFH-KMH <Samuel.Kemp@sfh-tr.nhs.uk>
Fri 15/02/2013, 14:50
You; Sonia Gill - Clinical Audit Support Officer - SFH-KMH (Sonia.Gill@sfh-tr.nhs.uk); Sushma Dhar-Munshi - Opthalmic Surgeon -KMH (Sushma.Dhar-Munshi@sfh-tr.nhs.uk); +1 more

Reply

Dear Sonia and Craig,

This does seem to be more research than audit. We'll discuss it in our R&I meeting next week. It won't need ethics as it is all retrospective, but you should keep us updated with progress as we may be able to help if any issues arise.

I'll let you know the outcome of our discussions.

Best wishes,

Sam.

From: Sonia Gill - Clinical Audit Support Officer - SFH-KMH
Sent: 14 February 2013 14:03
To: Craig Wilde Wilde; Sushma Dhar-Munshi - Opthalmic Surgeon -KMH
Cc: Samuel Kemp - Consultant - SFH-KMH; Samantha Jones - Research Nurse Team Leader -SFH-KMH
Subject: RE: Audit application

Hi Craig, Your "clinical audit" proposal was reviewed at today's meeting by Mr Pickles, Chair of the Clinical Audit Committee.

We are not approving your project as a clinical audit as the project is not a clinical audit.

- Your project could fall under the heading of "research" or "quasi-research" although I am not sure. I have copied this to our Director of Research, Dr Kemp and our Research Team Leader Sam Jones so they are aware of the project and can provide guidance as to whether this is a research or not and if it is what steps you have to take to "register" this project. .
- Alternatively your project could be a "service practice review" – I have attached some national guidance on how to determine if this is the case.

Regards
Sonia

Sonia V. Gill (Ms.)
Clinical Audit Support Officer,
Sherwood Forest Hospitals NHS Foundation Trust,
Level 2, Trust Admin Building

7.2.1 Definitions of SD-OCT grading with illustrated examples.

Figure 11: SD-OCT example of subretinal choroidal neovascular membrane

A fusiform or dome shaped area of high reflectivity located within the subretinal space directly adjacent to the presumed RPE. This may or may not be associated with SRF, IRF, or haemorrhage. Subretinal haemorrhage was ruled out as the cause of this lesion by using point to point correlation between the SD-OCT B scan and the OCT photograph. This utilised PinPoint Registration software.

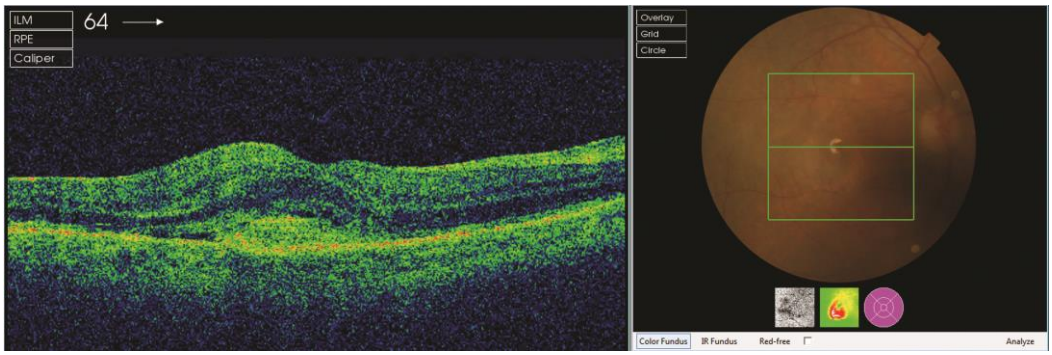


Figure 12: An example of the SD-OCT features of a fibrovascular PED

The presence of a non-drusenoid RPE elevation: a PED. If confluent drusen and a PED exist, a drusenoid PED was ruled out by the use of point to point correlation with the B scan and the OCT FP. The PED can range in appearance from a large dome shaped elevation of RPE with low reflectivity within and a visible Bruch's membrane, to an irregular and corrugated detachment that may be shallow with moderate reflectivity throughout or on the underside of the RPE. A PED was graded with or without SRF, IRF or haemorrhage.

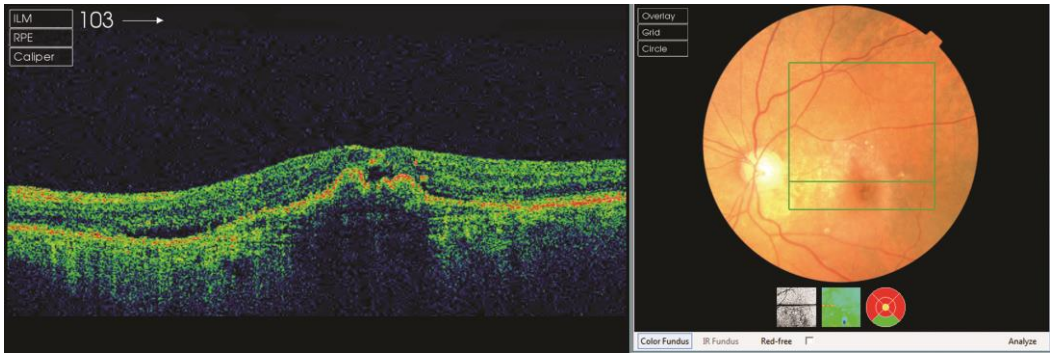


Figure 13: SD-OCT of a drusenoid PED with subretinal space

If a drusenoid PED exists and is associated with SRF/IRF/or diffuse retinal thickening over 250µm in the absence of other pathology to explain its origin.

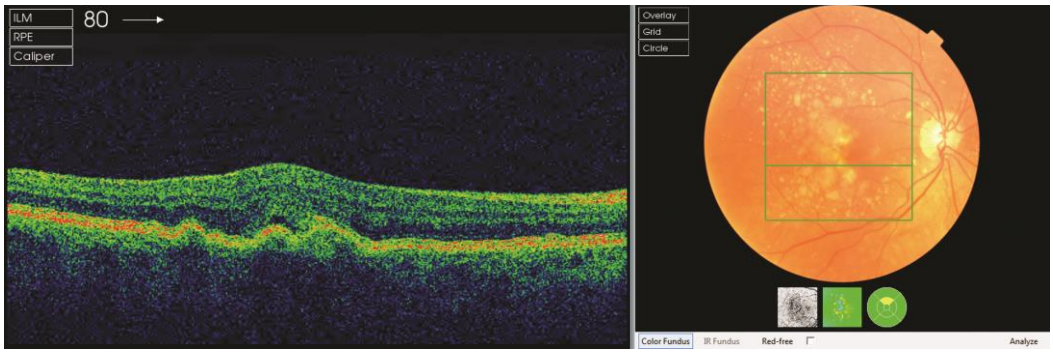


Figure 14: SD-OCT features of a peripapillary CNV

Features in keeping with a possible PPCNV or idiopathic polypoidal choroidal vasculopathy were also graded if there were: peripapillary exudate, haemorrhage, PED, retinal thickening or SRF/IRF that were due to no other identifiable ocular pathology and could be seen to approach the OCT macular grid from the optic disc/nasal direction.

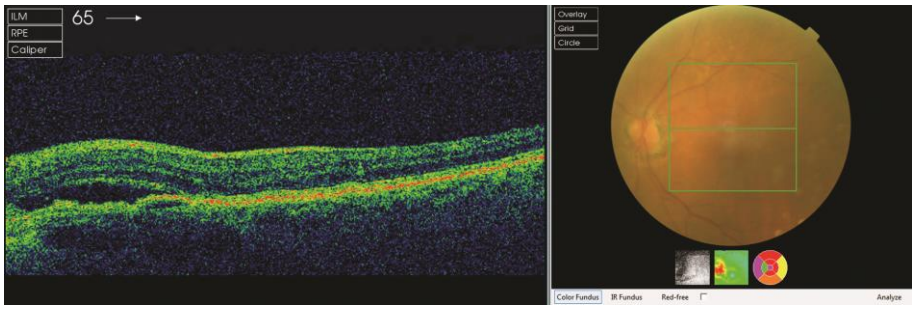
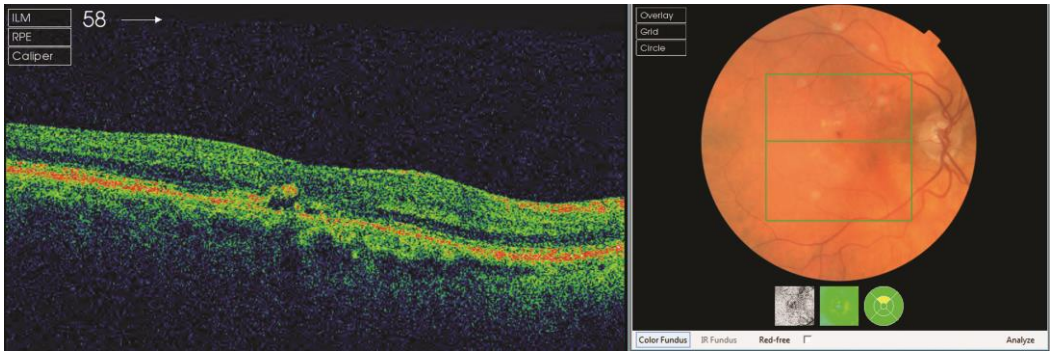


Figure 15: SD-OCT of disruption of the RPE with presumed IRF

The presence of intraretinal cystic spaces, SRF, or retinal thickening with no other identifiable aetiology to account for these changes was graded as questionable CNV. It has previously been demonstrated quantitatively that a late leak of undetermined source can occur without a PED.



Once all FFA and OCT images had been graded, if a disparity existed adjudication took place and any errors in grading corrected and the data cleaned.

7.2.2 Sample size calculation

Sensitivity and specificity is commonly used for screening and diagnostic tests. For a diagnostic test, an issue that needs to be addressed is to determine an adequate sample size that will yield a minimum value (for sensitivity and specificity), together with an adequate level of power and a sufficiently low level of type 1 error. Numerous studies have been conducted on sample size estimation for analysis of sensitivity and specificity. David et al (1991) emphasised the estimation of a minimum sample size required for a positive likelihood ratio with its corresponding confidence interval [240]. Nancy et al (1996) emphasised the importance of how to incorporate the value of prevalence of a disease into sample size calculation [241]. A further development was by Claes et al (2000) who introduced an approach for estimating sample sizes required when the true state or prevalence of the disease is unknown [242]. Sample size can be calculated using available formula as discussed here, or using commercially available programmes.

An alternative approach is to use sample size tables that are designed for the purpose of diagnostic research as published by Bujang et al (2016) [243]. They calculated the minimum sample size required for sensitivity and specificity tests, for screening and diagnosis, using Power Analysis and Sample Size (PASS) software (PASS 11 citation: Hintze J (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA). PASS is commercially available software that provides sample size tools

for various statistical tests and confidence interval scenarios. The group have published a series of tables of minimum sample sizes required, based on different values of prevalence of a disease and both sensitivity and specificity. In their calculations they fix the power to be at least 80% and the p-value is set to be less than 0.05.

We know from a pre-study sample, approximately 60% of new referrals to the AMD clinic at KMH have nAMD. We can therefore estimate the minimum sample size required by making reference to tables published by Bujang et al [243]. The prevalence of disease among new patients in the AMD clinic setting is 60%. Previous published reports of the sensitivity of SD-OCT for diagnosing nAMD have been high, in the region of over 90%. Therefore we can estimate a minimum sample of 385 eyes (including 231 with the disease) will be required to achieve a minimum power of 80% (actual power 0.816) in order to detect a change in the percentage value of sensitivity from 90% to 96%, with a target significance level of 0.048. This minimum sample size is also sufficient to detect a change in the value of specificity from 80% to 90%, which would only require a minimum sample of 268 subjects (including 161 with the disease).

This minimum sample size is also sufficient to detect a change in the value of specificity from 80.0% to 90.0% which will only require a minimum sample of 134 subjects (including 27 subjects having the disease). Our achieved sample size of 411 consecutive patients is therefore deemed adequate.

7.3 Results

A total of 411 patients (822 eyes) were referred to the macular clinic between the dates 02/2009 and 02/2013. All available FFA and OCT images were assessed. 346 eyes were removed as they had either no OCT, FFA or had images that were deemed upgradable. Included in this group were 6 eyes that had active CNV identified on both FFA and SD-OCT. However, there were insufficient FFA frames to allow CNV subtype classification and hence these eyes were removed. 476 eyes had both gradable FFA and SD-OCT. Of these, 198 eyes had no CNV identified with either FFA or OCT, including 19 eyes with chronic disciform scars, leaving 278 eyes that were graded as having CNV with either FA, OCT or both.

Within this group (of 278 eyes) the mean age of these patients was 80.6 years (SD 4.7), range 51-97. Of these 115 (41.4%) were male, 163 (58.6%) were female. A total of 32 patients presented with bilateral CNV with FFA. The main diagnostic classifications on FFA were: 27 eyes with classic no occult, 16 predominantly classic, 50 minimally classic, 129 occult and 9 PPCNV.

There were a total of 47 false positives with SD-OCT: a rate of 16.9%. Seven of these were diagnosed as disciform scars with FFA. These were graded as either subretinal membranes or PED's with SD-OCT. Thirty-seven of the remaining 40 false positives were graded for either: PED, IRF, SRF or retinal thickening. Reference to the FP changed none of our SD-OCT grades. There was only one false negative with the use of SD-OCT in the primary grading. This SD-OCT was

of a poor quality, and upon primary grading was believed to have no identifiable features of CNV visible on B scan or OCT photograph. Upon review of the FP peripapillary disc haemorrhages were noted and FFA revealed an occult lesion. However, during adjudication the SD-OCT was reviewed and nasal retinal thickening was in fact present but missed as part of a grading error. This haemorrhage was not visible on the OCT photograph. The sensitivity and specificity of SD-OCT alone for detecting CNV was 100% and 80% respectively. The positive predictive value (PPV) was 83%, with a negative predictive value (NPV) of 100%.

There were 77 eyes that had other ocular pathology identified as the primary diagnosis with no CNV associated; 46 of these eyes had no FFA but SD-OCT only. These diagnoses included 10 eyes with vitreomacular traction (VMT), 5 with a central retinal vein occlusion (CRVO), 11 with a branch retinal vein occlusion (BRVO), 12 with an epiretinal membrane (ERM), 22 eyes with adult adult-onset foveomacular vitelliform dystrophy (AFVD) and 17 eyes with other pathologies.

7.4 Discussion

SD-OCT is being increasingly used in clinical practice for the follow up of patients undergoing treatment with anti-VEGF. It provides a time efficient, non-invasive imaging tool that allows high resolution, pseudo-histological cross-sectional images of the retina, RPE and choroid. It is based on measuring the time delay of light reflected from each optical interface between structures of differing refractive index. Our study confirms that SD-OCT in comparison to the

reference standard of non-stereoscopic FFA is highly sensitive (100%) at detecting newly presenting nAMD in the setting of a specialist AMD clinic, where the investigations are interpreted by trained specialists. The specificity is comparable to those previously reported elsewhere [71, 74]. The sensitivity is higher than that obtained in some previous reports [71, 73, 74]. There are several explanations for this ranging from the use of different grading criteria, differing patient populations and, of course, diagnostic imaging technologies utilised. Sandhu et al (2005) reported the sensitivity with TD-OCT alone to be 96.4% [71]. The use of TD-OCT has been reported by others to frequently fail in the detection of abnormalities that are associated with fluorescein leakage from CNV on FFA [244] and a few studies have demonstrated SD-OCT to be more sensitive at detecting abnormalities associated with nAMD than TD-OCT [70, 76, 245]. This will, in part, explain the improved sensitivity with our results. The lower sensitivity reported by others such as Khurana et al (2010) [76] may reflect the study population; in that particular example 80% of assessed eyes had previously been treated with anti-VEGF. Another publication investigating the sensitivity in a setting of new patients referred for suspected wet AMD also reported a sensitivity of 100% [72]. Other differences in reported sensitivities may reflect the variety of ways that OCT images are graded and the inconsistency of definitions used for diagnosis. An example is the AMD Doc Study [73] that reported a sensitivity of 69% in the detection of conversion to nAMD in high risk eyes. The criteria used for the detection of CNV with SD-OCT primarily involved assessing retinal changes that occurred secondary to a breakdown in the outer

blood-retinal barrier such as the presence of sub retinal fluid, a 10% increase in retinal thickening or intraretinal cystic abnormalities. It has previously been demonstrated that second eye conversion to nAMD is unpredictable in only a small number of patients (12%). In the majority of cases, changes such as IRF or SRF were preceded by the development of RPE elevation [246]. Other studies like ours and that by Sandhu et al [71] have graded OCT images for the morphological features of the fibrovascular complex [244, 247] in addition to their exudative consequences of retinal thickening and oedema as opposed to just the latter. 5% of eyes in our study had no SRF or IRF but yet had an occult lesion on FFA. In all of these cases SD-OCT demonstrated a PED. This highlights the importance of grading PEDs in the absence of IRF/SRF to avoid unnecessary false negatives. However, it is accepted that the mere presence of PEDs does not imply an active CNV.

In our study during the primary grading there was only one false negative with the use of SD-OCT, which on adjudication turned out to be a grading error as explained earlier. This highlights another possible limitation of SD-OCT for the imaging of nAMD. Given the small field (6mm by 6mm) used for routine scans of the macular area, eccentric pathology and peripapillary lesions could be missed that would otherwise be detected with standard protocols within the larger 35° field of FFA. In the future as larger SD-OCT fields become more routinely used this problem may be overcome. The Topcon 3D OCT 1000 used in this study can image an area of 8.2mm which would have included both the disc and

the macula area and may have prevented this grading error. However, the imaging protocol did not include the disc and nasal peripapillary zones.

The high false positive rate represents difficulty in correctly identifying areas of hyper reflectivity on SD-OCT that represent active CNV, and distinguishing them from those that represent inactive gliosis, particularly in the setting of chronic lesions. SD-OCT, not unexpectedly, seems to allow easy identification of structural changes that indicate there has been a previous or currently active CNV using SD-OCT. However, it is unable to determine whether the fluid detected is from an active CNV at diagnosis. Furthermore, other causes of intraretinal cysts or fluid do occur and may confound diagnosis of CNV based purely on the presence of such spaces. Another possible explanation for some of these possible cases is that obscuring lesions such as a staining scar could have covered and masked any leak below on FFA.

The study demonstrates that in the setting of an AMD clinic, where images are being carefully reviewed by trained specialists, SD-OCT has a high PPV (83%). It has to be noted that the prevalence of nAMD in these patients was high. It was a clinic where patients were being referred in with a high index of suspicion of already having the disease. The prevalence of nAMD cases out of the total of referrals (among those with gradable images) was 58.4%. It is known that the PPV of a diagnostic test increases in situations where there is a high prevalence. The NPV was very high (100%), secondary to there being no false negative cases. Again, this NPV reflects the strict application of a detailed grading protocol that was developed in a way to avoid false negative cases as a priority.

It is acknowledged that the sensitivity, specificity, PPV and NPV of SD-OCT may change if tested in populations that are different to the one in the study, especially if the spectrum of disease varies. For example, in cases of more severe disease, it will be more likely to make a diagnosis and hence sensitivity will increase.

The cause of the discrepancies where SD-OCT demonstrates a PED without disruption of the RPE, or the presence of SRF/IRF is not known at the present time and definitely warrants further investigation. The association of subretinal space, presumed as SRF with PED may represent nothing more than potential space and contain no leaking fluid at all. The presence of intraretinal cysts may represent intraretinal changes secondary to chronic disease processes other than nAMD such as retinal dystrophy or other degeneration.

Alternatively, although unlikely, the findings may reflect SD-SOCT as being a more sensitive imaging modality than the presumed gold standard and these changes may in some eyes represent the very early structural changes that occur prior to a detectable leak with FFA[246]. In addition, the FFAs were limited to 10 minutes as in standard practice so that there is chance that late leakage of undetermined origin is missed.

Chapter 8: False positive diagnosis using spectral domain optical coherence tomography for neovascular age-related macular degeneration: an exploratory study

8.1 Introduction

AMD is the leading cause of visual impairment in elderly patients in developed countries [3]. A report from the WHO global eye disease survey estimated that 14 million people are blind or severely visually impaired due to AMD with 30-50 million people being affected worldwide [2]. Its prevalence has been reported across a range of populations with a recent cross-sectional study in the UK demonstrating a prevalence of 4.3% in the over 65-year age group, increasing to a maximum of 21.2% in individuals aged 90 years and over (Wilde et al BEAP). The two main phenotypes of AMD are GA, characterised by loss of the choriocapillaris, and the overlying RPE and photoreceptors, and nAMD signalled by the development of CNV from the inner choroid. There are currently no treatments for GA. Despite treatments with anti-VEGF therapies for nAMD, the number of individuals with sight loss secondary to AMD in the UK is expected to rise over the next decade as the population ages.

The diagnostic gold standard for nAMD is stereoscopic FFA [248] and is currently recommended by the Royal College of Ophthalmologists in the UK as a requirement for confirming diagnosis of CNV before treatment commencement (Chakravarthy college guidelines). OCT is now a commonly used to supplement FFA in the diagnosis, and monitoring of nAMD patients undergoing treatment

with anti-VEGFs [249]. We recently reported that SD-OCT when compared to the reference standard of FFA is a highly sensitive test. However, the use of SD-OCT in diagnosis in the setting of a specialist AMD clinic was limited by its specificity of 80.8%, and in our recent series a false positive rate of 16.9%[209]. FFA has well-recognised grading protocols that have widespread use in clinical practice, such as that used in the Macular Photocoagulation Study [77]. Currently, there are no internationally recognised grading systems for SD-OCT of nAMD, and there is a paucity of publications regarding the frequency of diagnostic errors encountered with its use[209]. Fewer publications exist that explore the reasons behind the assignment of a false positive diagnosis in nAMD. As nAMD is a chronic and progressive disease, with significant costs involved in its treatment, accurate diagnosis is of crucial importance to prevent unnecessary interventions. In this current report, we reviewed all of the false positive cases generated with the use of SD-OCT in our previously reported series, and the disposition of these eyes. (Wilde et al, 2015) The report investigates the SD-OCT findings behind the assignment of false positive diagnoses, and explored the potential diagnostic pitfalls. These findings will be valuable for any ophthalmologist who regularly uses SD-OCT alone for diagnosis or for situations where FFA is impractical or not possible.

8.2 Materials and Methods

The full details of the study cohort, imaging procedures and definitions used are published elsewhere[209]. Briefly, a retrospective review of all SD-OCT, FP and FFA of 411 consecutive patients (822 eyes) that were referred to a rapid access

macular clinic were reviewed. All patients were over 50 years of age, and suspected of having nAMD (by optometrists, general practitioners or ophthalmologists) and had symptoms of reduced vision, metamorphopsia or signs suggestive of nAMD as determined by the referring clinician. In total after exclusions for ungradable images, 231 eyes were diagnosed as having CNV with FFA. There were a total of 47 eyes with graded as false positives with SD-OCT: a rate of 16.9%. All available images for these 47 eyes were reviewed by two ophthalmologists with appropriate experience in AMD image grading (CW and WA).

All sequential SD-OCT images available for the 47 eyes were reviewed and graded for features of AMD as per the original study protocol [209]. All corresponding non-stereoscopic FP (35-degree field two, centred on the fovea) were reviewed and graded using the International Classification system[43] and assigned a modified Rotterdam grade for the stage of AMD at presentation. All FFA were reviewed and the absence of active nAMD confirmed. In addition, an alternative diagnosis was assigned to each of these eyes. A retrospective review of all patient case notes was performed. Symptoms at presentation, if any, were recorded. The clinic diagnosis for each eye along with treatment, if any was recorded. The Topcon 3D OCT-1000 instrument viewer programme was used for OCT image analysis. Central retinal thickness was measured at presentation using a manual calliper. The mean thickness of the central circle of the ETDRS grid was recorded at presentation. The vertical height and length of any PED was measured. If the patient received treatment, these measurements were

repeated post-treatment and a subjective text description of the perceived response to treatment recorded. LogMAR visual acuity (VA) was recorded at presentation and at each visit to clinic thereafter.

8.3 Results

The mean age of false positive eyes was 82.3 years (range 64.3-94.2) with 33 (67%) being female. A total of 37 (78.7%) of the false positive patients were symptomatic at their initial attendance of the macular clinic, reporting either metamorphopsia or reduced VA. Table 24 summarises the results of FP grading. The majority of SD-OCT false positive diagnoses (41 eyes, 87.2%) occurred in eyes that had pigmentary changes; either RPE hyperpigmentation, hypopigmentation, or both, and GA (Rotterdam stages 1b, 2b, 3 or 4a). All 3 eyes graded as having nAMD/Stage 4b had disciform scars on FP in the central foveal area.

Most (46%) of false positive diagnoses were attributable to the presence of a non-drusenoid PED as shown in Table 25. On SD-OCT the RPE elevation was felt not to correspond with visible drusen using point to point correlation of the OCT pseudocolour image in the absence of frank GA. Eyes identified as having GA/grade 4a on non-stereoscopic FP were the only exception to this trend, with 70.6% of SD-OCT images demonstrating both GA and RPE elevation. Almost 40% of eyes identified as having SD-OCT features in keeping with a diagnosis of nAMD had identifiable GA on FP when evaluated. As shown in Table 25, the vast majority of these eyes (88.2%) demonstrated elevation of the RPE on SD-OCT,

and 70.6% had areas of GA identified using SD-OCT (as defined by either a round or oval area of RPE loss with visible choroidal vessels on the pseudocolour image that corresponded to a well-defined area of increased OCT signal transmission below the RPE and a thinning or loss of the RPE). In 82.2% of these eyes the SD-OCT demonstrated IRF, SRF or both. A total of 15 false positive eyes that at presentation had evidence of GA on SD-OCT had associated elevation of the RPE in either the area of GA, as identified with FP, or in the peri-lesional area. Small areas of presumed SRF or IRF were commonly associated with this finding (86.7%). In several of the eyes with RPE elevation and GA, the GA extended laterally into the area of RPE elevation over time, raising the possibility that the RPE elevation may be a marker for GA progression or development. Table 26 illustrates that the majority of false positive diagnoses were associated with the presence of presumed IRF, SRF or both.

In 14 eyes (29.8%) the early FFA run was performed on the contralateral eye to the SD-OCT false positive. The non-stereoscopic FFA generally had large areas of uneven hyperfluorescence secondary to rarefaction and hypopigmentation of the RPE over drusen and hypofluorescent foci secondary to hyperpigmentation. This gave a stippled appearance that with the SD-OCT features may have resulted in a presumptive diagnosis of an occult CNV. Late staining of regressing drusen was also noted on FFA. SD-OCT false positives were assigned into one of five broad groups (angiographic) that best explained the reason behind their wrong grading. These groupings and their frequencies were: 1- a non-drusenoid

PED without atrophy at presentation (22 eyes, 46.8%); 2- established GA with intra-lesional or peri-lesional PED (15, 31.9%); 3-disciform scar (4, 8.5%); 4-intraretinal fluid (IRF) or SRF of no known aetiology (5, 10.6%); or 5 other diagnoses (1, 2.1%).

The majority (26 eyes, 57.4%) of SD-OCT false positives received treatment with intravitreal ranibizumab and therefore were felt to have active CNV at presentation, despite the absence of leakage on the FFA at presentation. Only 21 (44.7%) of SD-OCT false positive eyes were not treated. Treatment was given in 11.3% of eyes (26 eyes out of 231 true positives) that may be considered inappropriate.

The mean number of injections per patient delivered to the SD-OCT false positive subgroup was 9.1 (range 1-22). 38% of the false positive eyes that were clinically treated received 4 injections or less. The mean duration of follow up of patients within this group was 27.4 months. The average number of intravitreal injections/year within this group was 4.0. For the 26 individuals that were treated, the mean visual acuity was reduced by -1.6 letters.

Table 24: Colour fundus photograph modified Rotterdam grade for AMD stage for all SD-OCT false positive eyes.

Rotterdam Grade	Description	Number	Percentage
0a	Normal-no signs of AMD at all	2	4.3
0b	<10 hard drusen<63µm in size	0	0
1a	≥10 hard drusen or any soft distinct drusen ≥63µm	1	2.1
1b	Pigmentary abnormalities only, or with hard drusen 63µm in size, no soft drusen	12	25.5
2a	Soft indistinct drusen ≥125µm in size or reticular drusen only	0	0
2b	Soft distinct drusen ≥63µm in size with pigmentary abnormalities	10	21.3
3	Soft indistinct drusen ≥125µm with pigmentary abnormalities	2	4.3
4a	GA	17	36.2
4b	nAMD	3	6.4
4c	PPCNV	0	0
7	Other macular disease	0	0

Table 25: Correlation between SD-OCT false positive subgroups and FP Rotterdam grades.

SD-OCT false positive subgroups: 1- a non-drusenoid PED without atrophy, 2- atrophy with intralesional or perilesional PED, 3-disciform scar, 4- IRF or SRF of no known aetiology, 5 other diagnoses.

Rotterdam Grade	SD-OCT false positive subgroups: number (percentage)				
	1	2	3	4	5
0a	1 (50)	0	0	1 (50)	0
1a	1 (100)	0	0	0	0
1b	8 (66.7)	1 (8.3)	1 (8.3)	1 (8.3)	1 (8.3)
2b	8 (80)	2 (20)	0	0	0
3	1 (50)	0	0	1 (50)	0
4a	3 (17.6)	12 (70.6)	1 (5.9)	1 (5.9)	0
4b	0	0	3 (100)	0	0
Total	22 (46)	15 (31.9)	5 (10.6)	4 (8.5)	1 (2.1)

8.4 Discussion

The reasons behind most (up to 46%) of our false positive diagnostic errors are easy to establish. Many represent a SD-OCT grading system that was written for the initial study [209] with the intention to purposefully achieve a very high sensitivity, with grading rules established to avoid false negative errors. One such rule was that if any PED did not correspond to obvious drusen on the pseudocolour image then a grade of questionable nAMD should be assigned, even in the absence of IRF or SRF; a finding that accounted for up to 31.8% of false positives (Table 27). Drusenoid PED's have long formed part of the clinical spectrum of AMD, after first being described by Caswell in 1985 [170] and many reports have established their risk of progression to either GA or nAMD [250-252]. Drusenoid PEDs are dynamic structures whose natural history has demonstrated they pose a significant risk (42%) of the development of advanced AMD within 5 years, including 19% progressing to GA and 23% to nAMD in the AREDS [171]. Others have demonstrated that their long term risk is even higher. Roquet et al demonstrated that in eyes with drusenoid PEDs, within ten years, GA and nAMD occurred in 75% and 25% respectively and was associated with a poor visual outcome [251]. Eyes with drusenoid PEDs that were large in size (over 2 disc diameters) or were associated with metamorphopsia at presentation, were more likely to progress to GA or nAMD within 2 years [251]. In the AREDS study, eyes that did not progress to advanced AMD demonstrated progressive anatomical changes characterised by a decreasing total drusen area

(secondary to regression), increasing drusen calcification and increased RPE hypopigmentary and hyperpigmentary changes [171]. Some non-drusenoid PEDs in our series most likely represent regressing drusenoid PED's, before the RPE flattens in the progression to frank GA.

SD-OCT images in eyes with GA are known to sometimes demonstrate cyst-like spaces in the inner nuclear layer [253] and a recent publication by Fleckenstein et al [60] demonstrated that eyes with a rapidly progressive form of GA exhibited separation of the RPE from Bruch's membrane in the junctional zone surrounding GA. Our findings, of RPE elevation and cyst-like spaces in areas of GA or peri-lesional areas, on SD-OCT supports the findings from these previous reports. Small areas of presumed SRF or IRF on SD-OCT clearly pose a potential for diagnostic errors and confusion with nAMD. In addition, our findings suggest that RPE elevation may be a precursor to GA progression or development over time.

The presence of GA and its classification seemed to pose a particular challenge in this series. The classification system for GA in current clinical practice is perhaps inadequate and currently is only well defined using either the WARMGS [27] or the International Classification System [43] using FP. GA is clearly a heterogeneous condition and the current classification systems fall short in recording and recognising all its features particularly as it often develops as a continuum from regressing drusenoid PD's. A newer multimodal classification system is likely required utilising FAF, SD-OCT and FFA.

An unexpected finding of this study is that 57% of SD-OCT false positive eyes were treated for nAMD, despite the absence of angiographic features of active CNV when reviewed under study conditions. The implication of this finding is that FFA interpretation within this cohort is associated with a high degree of diagnostic error. The likely explanation is that most false positive eyes (87.2%) had significant pigmentary changes being evident on FP that would have confounded the appropriate interpretation. SD-OCT images that will have been reviewed prior to FFA analysis were associated with RPE elevation in 77.9% of cases and 80.9% of OCT images demonstrating either IRF or SRF or both. It may be that the PEDs and fluid visible with SD-OCT imaging were misleading to the reviewing clinician, and introduced an element of bias. The large areas of uneven hyperfluorescence secondary to rarefaction and RPE hypopigmentation over drusen and hypofluorescent foci secondary to hyperpigmentation may have been classified as the 'stippled appearance' of late leakage on FFA. Late staining of regressing drusen could also contribute to these FFA being complicated in their appearance. This raises the importance of quality assurance procedures being integrated into macular clinics, including regular FFA meetings to discuss the initiation of treatment for new patients.

It is very difficult to know for sure the exact reasons behind most of the false positive eyes. Many of the problems arise from drusen and drusenoid PED's being dynamic structures and the SD-OCT representing a snapshot of this potentially changing pathology. The drusenoid PED's may regress and the PED

may or may not collapse. Patients with regressing drusenoid PED's may not be asymptomatic and as atrophy develops metamorphopsia may occur which can confound and bias the reviewing clinician. Alternatively, other diseases may explain the false positive cases. Central serous retinopathy (CSR) can occur in older individuals. Hirami et al published a series of 30 patients with CSR, in which 33% of subjects were 60 years of age or older [254]). Although some report that the two conditions have different OCT characteristics [255], differentiation with OCT alone could prove difficult. Although eyes with nAMD, when compared to those with acute CSR, have been shown to be more likely associated with the presence of intraretinal fluid, large PED's and thickening and irregularity of the highly reflective line, these features are not diagnostic. Other authors have reported this diagnostic overlap [255] and clinicians must be wary of CSR as a condition that can masquerade as nAMD, particularly in individuals over 50 years of age that may have co-morbid drusen. In our series no FFA features of CSR were identified. PEDs are known to occur in up to 63% of acute CSR [256], and IRF, although an unusual feature, has been reported to occur in chronic cases (lida, yannuzzi, retina, 2005, p1-7). PED are a more common feature in CSR than previously thought, with Velthoven et al also demonstrating detectable PED's in 52% of acute CSR and 100% of inactive cases [257].

Another major source of diagnostic error is the assumption that IRF or SRF in the presence of a PED reflects active CNV. This is simply not the case. Eyes with regressing drusenoid PEDs can get areas of fragmentation of the RPE and

pigment migration into the retina with intra-retinal hyper-reflective material. These changes may give a suggestion of IRF when they may simply represent empty spaces. Similarly, regressing drusenoid PEDs can cause a mechanical lifting of the RPE at their margins. The majority of eyes with established GA have evidence of either IRF or SRF. Therefore, in the presence of GA, these findings should be considered as non-specific even if there is a peri-lesional PED. The reviewing clinician must use FFA to rule out active leakage from a CNV.

Another cause of diagnostic error was observed in individuals with disciform scars. Like GA, they pose several problems for diagnosis with SD-OCT alone. Firstly, there is no recognised and universal definition using SD-OCT that adequately describes its heterogeneous features. Disciform scars can cause significant gliosis and form well defined subretinal hype-reflective lesions that, in some situations, resemble subretinal membranes. In others, the appearance may mimic a PED or may be associated with areas of atrophy. The finding of a PED with both gliosis and thinning of the RPE/ atrophy is not infrequent. It seems diagnostic errors are not limited to OCT, with 50% of our SD-OCT disciform scar false positive cases actually being erroneously treated by the attending clinician for classic membranes. We postulate several reasons for this. Often around disciform scars there is significant retinal atrophy, sometimes with a PED (that is partly atrophic). When a FFA is performed this appears as hyperfluorescence. The atrophy is often well defined with a clear margin that forms an almost lacy pattern. Within the atrophic area there appears to be a deviation away from the normal choroidal perfusion, with a dropout of small

choroidal vessels, and a prominence of larger vessels. It also appears these changes associated with central staining can be confused for a large classic lesion by the unwary.

In summary, this exploratory study re-affirms the importance of performing multimodal imaging for the diagnosis of nAMD. It highlights the major pitfalls for diagnostic error with SD-OCT often occur in association with likely resolving drusenoid PED's and eyes with developed or developing GA and disciform scars, where the appearance can be complicated. It highlights that in these cases FFA in clinical practice is often misinterpreted and also difficult to interpret with a high rate of diagnostic errors. The reviewing clinician must not allow earlier review of the SD-OCT to bias their subsequent review of the FFA. Newer multimodal classification systems are required to adequately describe AMD and particularly GA, as currently there appears to be a confusing disparity between the appearance on FP and OCT given the heterogeneity of the perilesional area of GA and other findings.

Table 26: Association of presumed SRF and IRF as identified using SD-OCT stratified for AMD stage as per Modified Rotterdam grade and SD-OCT subcategory.

Numbers (percentage)

Rotterdam Grade	IRF	SRF	Either IRF, SRF or both	Total
0a	0	2 (100)	2 (100)	2
1a	0	1 (100)	1 (100)	1
1b	3 (25)	7 (58.3)	8 (66.7)	12
2b	4 (40)	7 (70)	8 (80)	10
3	1 (50)	1 (50)	2 (100)	2
4a	10 (58.8)	9 (52)	14 (82.4)	17
4b	3 (100)	1 (33.3)	3 (100)	3
Total	21 (44.6)	28 (59.6)	38 (80.9)	47
SD-OCT subcategory				
1-a non-drusenoid PED without atrophy	6 (27.3)	13 (59.1)	15 (68.2)	22
2- atrophy with intralesional or perilesional PED	10 (66.7)	8 (53.3)	13 (86.7)	15
3- disciform scar	4 (100)	2 (50)	4 (100)	4
4- IRF or SRF of no known aetiology	1 (20)	4 (80)	5 (100)	5
5- other diagnosis	0	1 (100)	1 (100)	1
Total	21 (44.7)	28 (59.6)	38 (80.9)	47

Chapter 9: Prevalence of reticular pseudodrusen in newly presenting adult onset foveomacular vitelliform dystrophy

9.1 Introduction

RPD were first described by Mimoun et al in 1990 as a particular yellowish pattern in the fundus of patients with AMD that were more clearly visible with red-free or blue light [258]. Interest in them quickly increased as their importance was recognised when a strong association between RPD and AMD was observed in 1995, as Arnold et al [30] reported that two thirds of eyes with RPD had or would develop nAMD. Since then several groups have reported a high prevalence of RPD in eyes with both GA and nAMD [35, 55, 177, 179, 259]. It has been shown that 30-50% of eyes with RPD will progress to AMD within 5 years [55, 147] and RPD have been shown to increase the risk for the development of AMD 4-6 fold when compared to eyes with other stages of ARM only [37, 147]. In the BDES, RPD were demonstrated to confer a higher risk of visual impairment. New developments in retinal imaging technologies has allowed for their improved visualisation, revealing their location to be internal to the RPE [44, 180, 260].

AFVD was first described by Gass in 1974 as a 'peculiar foveomacular dystrophy' . Since this initial description, which used FFA, many groups have used a wide range of terminologies to describe this relatively uncommon macular disease. In some cases there has been lack of consensus with regard to the diagnostic criteria and pathogenesis of AFVD [262]. Previous terms used include adult vitelliform macular dystrophy [263, 264], adult vitelliform macular degeneration [265,

[266] and pseudovitelliform macular degeneration [267] among others. AFVD has historically been included among a spectrum of pattern dystrophies such as reticular dystrophy of the RPE and butterfly-shaped pigment dystrophy [262, 268] and there can be significant overlap between these heterogeneous groups. There appears to be a wide range of phenotypes for AFVD, and although there is significant variability in the age of onset, patients generally remain asymptomatic until at least the fifth decade. Whilst the course of the disease may be benign for many, others can develop severe visual symptoms from the deposition of yellowish subretinal material within the macula which can lead to RPE alterations, atrophy, or the development of CNV [269].

The pathophysiological mechanisms underlying the formation of both RPD and AFVD have not been fully elucidated. To date, only a minority of AFVD cases have known genetic defects, and most cases appear to be sporadic [262]. Different genotypes can have their own typical phenotypic features and ages of presentation. The PRH2 gene has been linked to AFVD and has been shown to account for approximately 2% to 18% of patients in cohorts to date [270, 271]. IMPG1 and IMPG2 are two new causal genes for AFVD, identified in 8% of families in a recently published cohort [272] and in these cases the vitelliform dystrophies were characterised by late-onset moderate visual impairment and the frequent (69% of patients) association with drusen-like lesions [271] in the macula. Limited genetic studies are currently available regarding RPD. Puche et al evaluated 4 genes with known associations with AMD (ARMS2/HTRA1, apolipoprotein E, C3 and CFH) and found all of the genotypes studied were

similarly observed in patients with and without RPD. The current evidence seems to suggest that RPD occur in the same genotype and epidemiological background as AMD [203]. A recent publication by Gliem et al [273] has also reported a high prevalence (52%) of RPD in eyes with pseudoxanthoma elasticum (PXE). This observation is an interesting one in that PXE is normally an autosomal recessive genetic disease with an established pathophysiology. It is associated with a mutation in the ABCC6 gene with a corresponding phenotype that involves calcification and fragmentation of connective tissue rich in elastic fibers, such as the Bruch's membrane(BM) of the eye [274]. Interestingly the mean age of the patients with PXE and RPD in that report was 48.6 years, which is considerably lower than that for isolated RPD. The authors surmised that this association revealed that RPD occurred not only in individuals with AMD and of advanced age but may be the end result of dysfunction of the BM. More recently a similar association between RPD and Sorsby Fundus Dystrophy has been reported [275].

Both RPD [276] and cuticular drusen [277, 278] have previously been associated with vitelliform type dystrophies. There is, however, very limited literature available on the association of RPD and AFVD. To the best of our knowledge, Zweifel et al were the only group that has reported the association other than in case reports [276]. This was, however, a small study with 7 eyes of 6 patients only, and did not report the prevalence of RPD within eyes presenting consecutively with AFVD. It is, therefore, difficult to assess the strength of the association. Knowledge of the prevalence of RPD in eyes with newly presenting AFVD will

allow comparison to similar populations with other diseases such as nAMD. Any significant differences may give important information regarding possible genetic associations between the two conditions and may lead to a better understanding of any pathophysiological mechanisms involved with the aetiology of the conditions.

We report a consecutive case series of newly diagnosed AFVD and report the associated prevalence of RPD within this cohort. Within our defined population, we also evaluated the prevalence of RPD within eyes with newly presenting GA and eyes with angioid streaks to allow direct comparisons between prevalence rates.

9.2 Materials and Methods

All the different multimodal imaging for consecutive patients that were referred to a rapid access Macular Clinic in Kings Mill Hospital, Sutton-in-Ashfield, over a 4-year period (February 2009 to February 2013) with newly presenting AFVD were retrospectively reviewed. All cases of AFVD were identified from new patients referred to the macular clinic at the KMH. Out of a total of 411 new referrals, in which all available imaging modalities was reviewed, AFVD was diagnosed in 15 patients, giving a prevalence of 3.65% in new referrals to the clinic.

Inclusion criteria were all new patients with a clinical diagnosis of AFVD, confirmed by the presence of a sub-foveal, round, yellowish and predominantly homogenous lesion with the corresponding presence of hyper-reflective sub-retinal material on SD-OCT as illustrated in

Figure 16. Cuticular drusen were specifically differentiated from RPD by their sub-RPE location on SD-OCT imaging and their intense starry-sky appearance on FFA, when available. All patients underwent a complete ophthalmological assessment at their clinic attendance, including slit lamp examination, SD-OCT, FP and FFA (as indicated) to rule out CNV. Exclusion criteria included all AFVD eyes with comorbid CNV, established GA, other macular pathologies or poor quality images that would confound grading. All available SD-OCT, FP, red-free and blue light images and FFA were graded for the presence of RPD. Since there is no recognised gold standard imaging method for RPD, their presence was considered definite if they were detected with either the SD-OCT or the FP. SD-OCT images were acquired using the 3D OCT-1000 instrument (Topcon, Tokyo, Japan). The field of view used was a 6mm by 6mm area centered on the fovea. A raster scan consisting of a total of 128 frames each made up of 512 axial scans was performed for each eye within this field. All 128 frames within each raster scan were reviewed for the presence of RPD. RPD were considered present if there were five or more discrete hyper-reflective collections in the sub-retinal space that were sufficient to alter the contour of the presumed inner segment-outer segment junction, as previously described by others [44], and illustrated in

Figure 17. In addition, some individuals had further imaging with the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). With FP, RPD were considered present if there was a definite reticular pattern of round or oval yellow-white lesions that joined to form an ill-defined network of broad, interlacing ribbons. An example is shown in

Figure 18. The FP was initially graded without digital alteration and subsequently graded with adjustment to the red-free and the blue channels of the fundus photograph using the IMAGEnet i-base program.

For all eyes identified as having RPD and AFVD, the topographical distribution of RPD was evaluated. In brief, an ETDRS Grid was placed on all available FP or SD-OCT images and the presence of RPD was recorded for all 9 subfields, along with outside the grid and nasal to the optic disc, as described elsewhere [37].The ETDRS grid was opened and initially aligned automatically within either the IMAGEnet i-base program (for the review of FP) or within the Topcon 3D OCT-1000 viewer program, for OCT image analysis. If required, manually repositioning was performed.

All consecutive newly presenting cases of pure GA and all eyes with identifiable angioid streaks were also reviewed and graded for the presence of RPD. GA was diagnosed if there was a sharply demarcated area of RPE loss that was at least 175µm in diameter within the ETDRS grid on FP. The lesion should be roughly round or oval in shape, with at least 2 of the following features: scalloped

edges, visible choroidal vessels that are more prominent than in the surrounding areas and well defined margins in-keeping with the clarity of the fundus photograph. If any part of the area of GA was contiguous with peripapillary atrophy, then it was not graded as GA. Any eye with combined GA and nAMD was graded as the latter. Angioid streaks were diagnosed of FP if there were irregular orange-red lines radiating from the optic nerve head and passing peripherally across the retina, corresponding to breaks in Bruch's membrane. They would correspond to a window defect with FFA if performed.

All retinal images were graded by at least two ophthalmologists with appropriate experience in AMD grading (CW, MP, AL or WA). Side by side grading with immediate open adjudication was used. If disagreement between the two grading ophthalmologists occurred, or a finding was graded as questionable, adjudication by a third ophthalmologist was performed.

Eyes with newly presenting AFVD were divided into those with and without RPD. The two groups were compared for gender using the Fisher's exact test. The mean ages of the two groups were calculated. Subsequent to this analysis all eyes with newly presenting GA were also reviewed to allow comparison regarding the strength of association with RPD between the various pathologies within the defined population.

9.3 Results

A total of 15 consecutive patients were identified as having newly diagnosed AFVD. All these individuals were Caucasian with 11 (73.3%) of these patients being female. The mean age at presentation was 77.3 years, with a standard deviation (SD) of 6.3 years. One patient had a disciform scar in the contralateral eye. Excluding this individual, AFVD was a bilateral finding in 12 out of 14 patients (85.7%). In total, a cohort of 27 eyes was identified with AFVD and ten (37%) of these eyes had RPD. Six patients (40%) with AFVD had RPD. Within this cohort RPD were a bilateral finding in 100% of patients. The mean age of patients presenting with AFVD and having comorbid RPD was 80.5 years (SD 3.7), while the mean age of patients presenting with AFVD without RPD was 75.1 years (SD 7.0). This age difference did not reach statistical significance (with an unpaired t-test) p=0.1. There were no males with AFVD that were identified as having RPD. However, Fisher's exact test did not show this difference to be statistically significant (p value=0.10).

Over the same time period, 92 eyes presented with newly documented GA. Twenty-three (23) of these eyes (25.0%) had RPD present. The mean age of eyes presenting with GA was 82.1 years (SD 8.5), and within this group, eyes with GA and comorbid RPD had a higher mean age than those without RPD, (83.1 vs 81.5 years). This difference was not statistically significant (unpaired t-test, P=0.45). Eyes presenting with GA and RPD were more likely to be female (87% vs 13 %); this gender difference reached statistical significance (p=0.01).

Twelve (12) patients also presented with identifiable angioid streaks with 4 (36.4%) having RPD in at least one eye. The mean age of these patients was 79.5

years. Within this group, 9 (75%) patients also had a newly presenting CNV, disciform scar and/or GA.

The topographical distribution or RPD in eyes with newly presenting AFVD is shown in Figure 19.

3 Discussion

There is very limited data available on the prevalence of RPD from large population based epidemiological studies [37, 147]. It is known that RPD are an infrequent finding, particularly in younger individuals. In the BMES their prevalence was reported to be 1.95% in Australian subjects aged 49 years of age and older, with a 15-year cumulative incidence of 4.0% (n=95) [147]. In the younger American population (43 years and over) of BDES, the reported prevalence of RPD was lower at 0.7%. The overall 15-year incidence was, however, similar at 3% [37]. It is well documented that their prevalence increases with age and was reported to be as high as 6.6% in the 75-86 year age group in the BDES [37]. The highest 15-year cumulative incidence in the BMES (4.9%) was reported in the 75 years and over age-range. Finger et al demonstrated persons with definite RPD were older than those with large drusen (over 125µm) and no RPD (76±4 vs 68±9) [58]. The 40% prevalence of RPD in consecutive patients with newly presenting AFVD in this series is much higher than reported in otherwise healthy individuals from any of the reported large epidemiological studies. To the best of our knowledge, this is the first time the prevalence of RPD has been described within an AFVD population.

Also notable in the present study was the gender specific prevalence of RPD in females with newly diagnosed AFVD. This gender disparity did not reach a level of statistical significance, probably because of the small sample size. This finding is consistent with previous reports of RPD having a female preponderance. When adjusted for age, their 15-year cumulative incidence has been shown within the BMES to be twice more likely in females compared to males (5.6% vs 2.2%) [147] [30, 35, 147, 176, 179]. Arnold et al reported 87% of patients with RPD were female [30] and Klein et al demonstrated the prevalence of RPD to be 2.5 times higher in woman within normal subjects [37]. Similarly, in the BMES, after adjusting for age, Joachim et al demonstrated the 15-year cumulative incidence of RPD was twice as likely in females [147]. In the cohort of 6 patients reported by Zweifel et al, 4 individuals with AFVD and RPD were female [276] but their series was not consecutive. Gliem et al reported no difference in sex distribution in their study on the association between RPD and PXE [147].

The reasons for this gender disparity remain unknown. In the BDES, Klein et al reported a 54% decreased survival rate in subjects with RPD at baseline [37]. There is increasing evidence to suggest an association between RPD and cardiovascular problems. Boddu et al [57] reports patients with RPD are more likely to be hypertensive than those with large soft drusen. In the Melbourne Collaborative Cohort Study, Finger et al found that RPD were associated with a moderately elevated systolic blood pressure, current smoking and a trend for an association with a history of myocardial infarction or stroke [58]. The association was, however, reported to disappear in their multivariate analysis, indicating a

modest effect size. In the BDES, while controlling for age, RPD were associated not only with being female (OR 2.67, 95% CI 1.16, 6.17, p=0.02) but also having a lower income (OR per lower income group 1.75, 95% CI 1.16, 2.62, p=0.007), higher body Mass index (OR per 1kg/m^2 1.08, 95% CI 1.02, 1.15, p=0.006), and more pack years smoked (OR 35 or more pack years smoked vs none 2.61, 95% CI 1.17, 5.85, p=0.02). In the BMES, Tan et al found that high-density lipopreoteins (HDL) were inversely related to the incidence of late AMD [59]. A history of any cardiovascular disease, including stroke, myocardial infarction or angina was also associated with incident early AMD and incident soft or reticular drusen [59]. These associations with cardiovascular disease could explain the preponderance of RPD in older females, if young males with RPD were at increased risk of mortality from cardiac disease. This question clearly warrants further study.

The existing prevalence studies may have underestimated the frequency of RPD to some degree because of the sole use of FP in their grading. It is known that newer imaging modalities such as SD-OCT, FAF and IR have led to improvements in the diagnostic accuracy of RPD [52, 176, 200] and multimodal imaging is now becoming the norm for RPD identification. This discrepancy in imaging sensitivities would obviously not explain this significant disparity in prevalence. The association between AFVD and RPD appears to be stronger than that for GA, as in our series, RPD were present in only 25% of eyes presenting with GA. However, it is well documented that RPD can fade with the development of GA

and CNV. It is, therefore, possible that some patients with GA may have had RPD which subsequently regressed.

We acknowledge, as pointed out by others [279], that although the SDD identified resemble closely, and share the phenotypic and demographic features of RPD, there remains a lack of histopathological and biochemical correlation similar to that published for RPD occurring in AMD [30, 184]. For this reason, some have recommended using the term 'RPD-like' lesions until such a histopathological correlation become available for pathologies other than AMD. Within our study however, the topographical distribution of the RPD-like lesions within eyes with AFVD had a distribution that was almost identical to that published by Klein et al, from the BDES population, with a significant preponderance of RPD within the inner and outer superior subfields along with outside the grid, particularly superiorly [37]. We believe that this is supportive of the suggestion that they are indeed the same as RPD seen within AMD populations.

The prevalence of RPD (36.5%) in our patients with identifiable angioid streaks is very similar to that reported by Gliem et al [273] in their prospective series of biopsy confirmed PXE patients. The mean age of patients is, however, considerably higher in our series (79.5years) compared to that of 48.6 years reported by Gliem et al [273]. It would be expected that given our much older population, the prevalence of RPD would be higher. The populations in the 2 studies obviously differ, however, in that the Gliem series consisted of patients with confirmed diagnosis of PXE either through genetic testing or through skin biopsy. Our patients had angioid streaks identified with no specific diagnosis

regarding their aetiology being made. However, it is likely some of these patients may have had PXE.

RPD have been reported by others to have a prevalence of around 40% in newly presenting AMD [176]. This present study confirms that RPD are not a finding restricted to eyes with AMD but rather a finding common among diseases where the pathophysiological mechanisms primarily involve damage to the BM and the RPE, whether genetic in origin or degenerative. Our study supports the concept that RPD appear to occur with high but variable frequencies in eyes with various retinal and macular pathologies, ranging from monogenetic diseases such as PXE (52% as published by Gliem et al), angioid streaks (36.5% in our series), to other more poorly defined conditions with a strong genetic component such as AFVD (40%). Their prevalence in eyes with GA is likely underestimated in our retrospective series at 25%, most likely because of the known tendency for RPD to regress with the expansion of GA [165].

Figure 16: An example of AFVD associated with RPD using the Heidelberg SD-OCT.

A sub-foveal, round, yellowish, and predominantly homogenous lesion corresponds to hyper reflective sub-retinal material on OCT. There is incidental vitreomacular adhesion. The IR image clearly identifies the RPD giving a target lesion appearance, with the center being brighter than its surroundings.


23 / 49
200 µm
200 µm
7/16/2015, OS
IR&OCT 30° ART [HS] ART(16) Q: 28
HEIDELBERG ENGINEERING

Figure 17: Heidelberg SD-OCT demonstrating discrete hyper-reflective collections in the subretinal space

The SDD cause undulation of the ellipsoid band. One appears to protrude towards the outer nuclear layer.

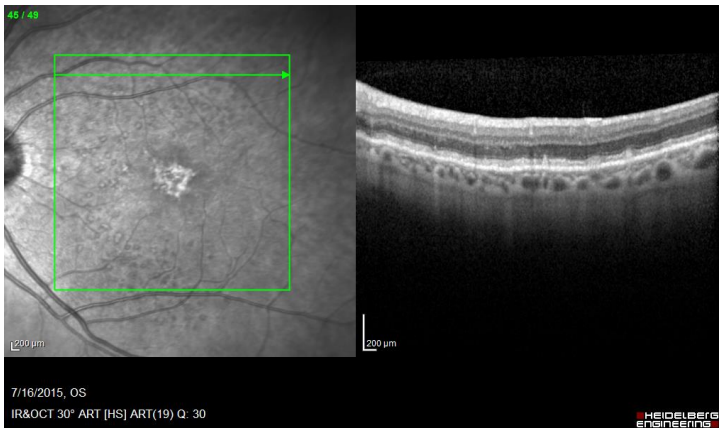


Figure 18: A colour fundus photograph demonstrating RPD.

There are multiple round and oval yellow-white lesions that join to form an ill-defined network of broad, interlacing ribbons that are predominantly located in the superotemporal location. There are both 'dot' and 'ribbon' types of RPD. This photo is for illustration purposes only. It has an incidental PPCNV, rather than vitelliform lesion. RPD can be subtle on FP.

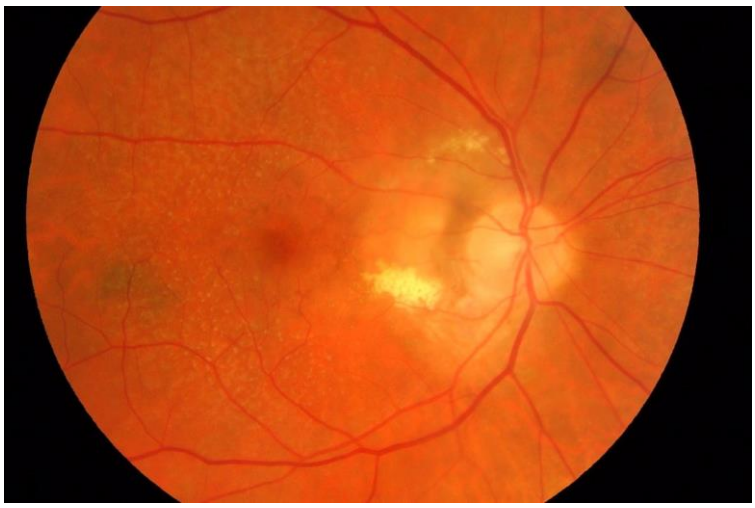
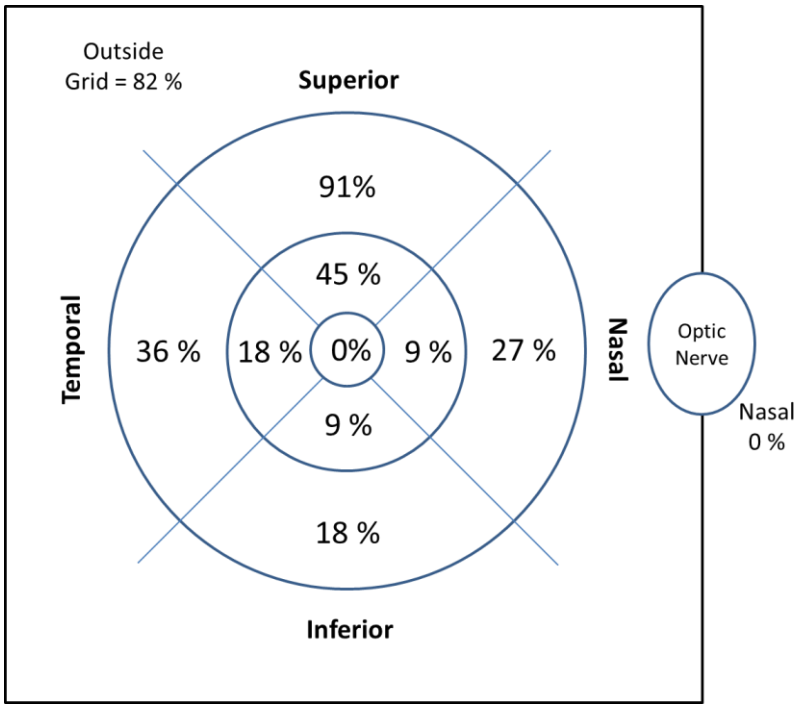


Figure 19 :Topographical representation of the fundus distribution of RPD in eyes with AFVD.

Eleven eyes were included as one eye was excluded given the presence of a disciform scar.



Chapter 10: Prevalence of reticular pseudodrusen in eyes with newly presenting neovascular age-related macular degeneration

10.1 Introduction

Across industrialised countries AMD is the most frequent cause of visual loss in older individuals [23, 36, 280]. The earlier stage changes in AMD are referred to as ARM. Visual impairment may result from the later stages of AMD, which manifests as either GA, or exudative/neovascular disease which implies the presence of a CNV [43]. Previous classification systems developed for epidemiological studies and clinical trials utilised FP as their single imaging modality [27, 40, 43]. These classification systems were based primarily on the assessment of drusen, and assessment of their margins (distinct or indistinct), size and substance (hard or soft) along with number, distribution and confluence, as well as the association of pigmentary changes. The association between drusen and GA or CNV has long been established and was first reported by Gass in 1973 [114]. These yellow sub-RPE lesions have become recognised as the hallmark of ARM. The presence of drusen (along with areas of focal hypopigmentation or hyperpigmentation) has come to signify an increased risk of progression to the more advanced stages of AMD and the associated potential for visual loss [107, 115].

A new phenotypic description within the spectrum of the already diverse ARM was added in 1990 with a publication by Mimoun et al [29], who described a particular yellowish pattern in the fundus of patients with AMD that was more

visible using red-free or blue light fundus photography. They termed the pattern 'les pseudodrusen visibles en luminere bleue', meaning 'pseudodrusen visible with blue light' [29]. The importance of this newly described fundus finding became clear when Arnold et al in 1995 observed that two-thirds of eyes with RPD had or developed CNV [30]. It became evident that RPD had clinical features and prognosis that were different from those of conventional drusen. Several publications have since revealed a high prevalence of RPD in eyes with AMD [35, 55, 88, 163, 176, 177, 204, 281].

There is only limited data available on the prevalence of RPD within large population based epidemiological studies [37, 147]. One reason for this is the previous misclassification of RPD as other forms of soft drusen, along with difficulties in their clear identification without the use of multimodal imaging. Furthermore, RPD were not specifically included in the international classification system for AMD [43]. Therefore, when present they would not have been differentiated from soft drusen using this international grading system [43]. The BDES by Klein et al [27] did record this phenotype as a separate entity named reticular drusen, using mydriatic FP. The reported prevalence and 15 year cumulative incidence in the over 43 age group were 0.7% and 3% respectively in the BDES [37].Their prevalence increased with age and is as high as 6.6% in the age range 75-86 years. [37] In the BDES, eyes with RPD were twice as likely to progress to AMD compared to those with soft drusen. RPD also conferred a higher risk of developing visual impairment. The actual prevalence of RPD within the community and eyes presenting with AMD may, however, have been

underestimated in some previous studies that have utilised FP alone in their grading. Newer imaging modalities such as SD-OCT, IR, FAF and confocal SLO have led to improvements in the diagnosis of RPD [34, 44, 52, 176, 178, 200]. The higher sensitivity and specificity for some of these imaging modalities has led to the assessment of RPD being based on multimodal imaging in the more recent publications. A limited number of previous publications available on the subject suggested a higher prevalence of RPD in eyes with retinal angiomatous proliferations (RAP), a particular nAMD phenotype [35].

We performed a retrospective review of all consecutive cases of newly diagnosed nAMD in our center in order to assess the association with RPD. We also assessed the different CNV lesion subtypes for any association with RPD.

10.2 Methods

A retrospective review of all SD-OCT, FP, and FFA of 202 consecutive patients that presented to a rapid access Macular Clinic in Kings Mill Hospital, Sutton-in-Ashfield, over a 4 year period (February 2009 to February 2013) was performed. Inclusion criteria were all patients over 50 years of age with active CNV in at least one eye during the period of interest. Exclusion criteria were patients that had either no SD-OCT or FP/FFA available for analysis or those where one or more imaging modality was deemed un-gradable. Patients with bilateral CNV were not excluded. Patients with CNV secondary to angioid streaks or chorioretinitis were excluded. No patients were excluded on the basis of their best corrected visual acuity. A small number may have had previous treatment with PDT or intravitreal injections of anti-VEGF agents at 6 months or more

previously. These were included as new cases of CNV. The refractive status of patients was not known, and as such, a small number of individuals may have been myopic but were not specifically excluded from the analysis.

FP was performed using the Topcon TRC-50DX, Type IA retinal camera (Topcon, Tokyo, Japan) combined with an attached Nikon D7000, 16.2 Megapixel camera. The operator was an experienced ophthalmic photographer. Bilateral 35° mydriatic non-stereoscopic photographs of field 2 (centered on the fovea) were reviewed using the IMAGEnet i-base system (Topcon, Tokyo, Japan). The standard FFA protocol included 35° images of the transit phase, mid phase and late phase up to 10 minutes. Images were all reviewed non-stereoscopically. SD-OCT images were acquired using the 3D OCT-1000 instrument (Topcon, Tokyo, Japan). The field of view used was a 6mm by 6mm area, centered on the fovea. A raster scan consisting of a total of 128 frames, each consisting of 512 axial scans was performed for each patient within this field. All 128 frames within each raster scan were individually reviewed for each patient for the presence of RPD covering a large macular area.

All images were graded by at least two ophthalmologists with appropriate experience in AMD image grading (CW, MP, AL or WA). Side by side grading with open discussion was used. The graders were blind to any patient clinical information such as history or visual acuity. If there was disagreement between the two grading ophthalmologists, then immediate open adjudication would take place. If agreement could not be reached or the 2 ophthalmologists were not 90% confident of their assigned grade, then adjudication by a third

ophthalmologist would occur. All SD-OCT images were reviewed without reference to the FP and vice versa. With FP, RPD were considered present if there was a definite reticular pattern of round or oval yellow-white lesions that joined to form an ill-defined network of broad, interlacing ribbons. An example is shown in Figure 20. The FP was initially graded without digital alteration and subsequently graded with adjustment to the red-free and the blue channels of the fundus photograph using the IMAGEnet i-base program. An example of the enhanced visualization of the RPD is demonstrated in Figure 21. Although the actual histological localisation of the lesions is controversial [30, 182], RPD were considered present on SD-OCT by the definite presence of five or more discrete hyper-reflective collections in the subretinal space that were sufficient to alter the contour of the presumed inner segment-outer segment junction [44] as shown in Figure 22.

FFA of CNV lesions were graded using the Modified Photocoagulation Study (MPS) grading protocol that was utilised in the treatment of AMD with photodynamic therapy (TAP) and verteporfin in photodynamic therapy (VIP) studies [237, 239]. Classic CNV was identified as an area of uniform and early (less than 30 seconds) hyperfluorescence that showed leakage throughout the mid and late phases. Occult CNV was identified by areas of increasing stippled hyperfluorescence that appeared in the mid and late phases of the FFA with a leak or a late leak of undetermined origin. FFA images were graded as either: classic, predominantly classic, minimally classic, occult, PPCNV, no CNV or other pathology. CNV was considered present on FA if classic or occult leakage was

detected. In addition to the above grading, images were assessed for the presence of a RAP as described by Yannuzzi et al [282].

Eyes with newly presenting nAMD were divided into those with and without RPD. The two groups were compared for gender, age, laterality of eye involved and bilateral presentation using the Fisher's exact test. The Chi-Squared test was used to assess the association of CNV subtypes with RPD. For RAP the Fisher exact test was used. All images of contralateral eyes (when available and gradable) of patients presenting with nAMD were also reviewed and graded using the methods described above.

We certify that all applicable institutional and governmental regulations were followed during this research.

10.3 Results

Our study cohort was composed of 202 consecutive patients presenting with nAMD. Twenty-nine individuals (14.4% of patients) presented with bilateral active CNV, whilst the other 173 had unilateral CNV. As such, a total of 231 consecutive eyes were studied, of whom 131 (56.7%) were in female patients. The mean age at presentation was 80.4 years, with standard deviation (SD) = 7.6 years. Of these, 51 eyes with CNV (22.1%) had identifiable RPD with one or more imaging method in that eye. Overall, 30.3% of patients with newly presenting CNV in either or both eyes had identifiable RPD. Where contralateral gradable images were available, RPD were a bilateral finding in 85.4% of patients. RPD were identified more commonly in the eyes of women than men (72.5% vs 27.5%), a difference that reached statistical significance (p=0.011). No

significant difference was noted between eyes with and without RPD with regard to age or eye involved (Table 27). Of the 29 patients (58 eyes) presenting with bilateral active CNV, 12 eyes (20.7%) had RPD. In the 173 eyes presenting with new onset of unilateral CNV, 39 (22.5%) had RPD. No association was found between the presence of RPD and bilateral presentation with active CNV (p=0.852). No association of RPD with CNV subtype was found. The association of RPD with the different FFA types of CNV are summarised inTable 28, and show that there is no particular association of RPD with any particular CNV subtype. Similarly, the presence of RAPs with RPD are summarised in

Table 29 and indicate no statistically significant association (p=0.688).

Images for 124 contralateral eyes to that with the CNV (unilateral) were available for analysis after exclusions. These exclusions were fifty-eight (58) eyes (of 29 patients) presenting with bilateral active CNVs, sixteen (16) contralateral eyes that had disciform scars (end stage CNV) which confounded adequate analysis, and another 33 contralateral eyes because the FFA, OCT or FP were either ungradable or not available. Thirty-nine (39) of the 124 available contralateral eyes (31.5%) had RPD, with an average age of 80.64 years (SD 8.73). A total of 32 contralateral eyes had GA (25.8%), with 13 of these (33.3%) having RPD present. Nineteen (19) contralateral eyes without RPD had GA. The association of GA and RPD did not reach statistical significance (p=0.268).

In the 155 eyes (67.1%) with newly presenting CNV there was identifiable drusen of ≥ 63µm size within the ETDRS grid. The prevalence of such drusen

increased to 96.1% in eyes with RPD. In addition, 12.6% of eyes with newly presenting CNV had confluent drusen.

In individuals that had detectable RPD in either eye, 48.6% had GA, active CNV, or a disciform scar in the contralateral eye. This occurrence was similar for individuals without RPD (44.7%).

The different imaging methods used in our study revealed different abilities to identify RPD. Of the total 51 eyes with CNV that had identifiable RPD with one or more imaging method, the RPD were identified in 66.7%, 78.4% and 90.2% using SD-OCT, FP and red free and/or the blue channel of FP respectively.

10.4 Discussion

RPD is now recognised as part of the AMD spectrum [35, 55, 88, 163, 176, 177, 204, 281]. The previously published rates of the occurrence of RPD in newly diagnosed nAMD vary amongst the different studies from 14 to24% [35, 283]. In the present study the prevalence of RPD in consecutive cases of newly presenting nAMD was high (22.1%). Cohen et al [35] evaluated images from a population in France (similar to that in our study) using colour, red free and blue light FP, and reported an almost identical rate of 24% of RPD. Ueda-arakawa et al [283] demonstrated that within a Japanese population with newly presenting nAMD the prevalence of RPD was lower at 14%. These differences could be explained by the different imaging modalities used as well as the inconsistency of grading definitions. The Japanese study, for instance, would only grade RPD as definitiely present if identified on two or more imaging modalities whist the Cohen study and the present one used presence on 1 or more imaging modality.

The study reported by Hogg et al [2014] suggested that RPD were best visualised by IR imaging which was not used in the present study. [19] Ethnicity, gender or age distributions of the various populations may also play a role in the prevalence of RPD. RPD are in the mainstay a bilateral finding, with 63% of cases identified in the BDES having them in both eyes [20]. In a 3 year prospective study, Pumariega et al [55] demonstrated that patients with unilateral CNV and large soft drusen in the fellow eye at baseline had a higher rate of progression to CNV if RPD were present as opposed to absent (44.7% vs 26.6%, p=0.002) [55]. The higher rate of RPD prevalence reported across prospective studies likely reflects the known tendency for RPD to fade with the development of CNV[55, 182]. It is possible that some of the patients in our study may well have had RPD prior to the development of their CNV, which subsequently regressed or were obscured by the developing CNV or its consequences such as haemorrhage or fluid accumulation. This would be more likely for large membranes at presentation.This difficulty in RPD identification and grading in eyes with active nAMD may explain why the prevalence of RPD was higher when available contralateral eyes were reviewed within our cohort (31.5% vs 22.1%).

In the present study, the proportion of females with newly diagnosed nAMD in the RPD group was higher than for males (72.5% vs 27.5%); this difference in gender reached statistical significance (p=0.011). This finding is consistent with that from previous reports [30, 35, 147, 176] although, to the best of our knowledge, this is the first time such a difference has reached a statistically significant level

within a population of newly incident nAMD. Arnold et al [30] found that 87% of patients with RPD were women, while Klein et al demonstrated that the prevalence of RPD was 2.5 times higher in women than men [37]. In the older Australian population, Joachim et al [147] demonstrated that after adjusting for age, the 15-year cumulative incidence of RPD was twice as likely in woman than men (5.6%[95% CI, 5.59-5.61] vs 2.2%[95% CI, 2.19-2.21]). Our findings further confirm the increased prevalence of RPD among women with nAMD. We found no difference between the ages of persons with or without RPD in our study. Others have suggested that patients with neovascular AMD and RPD were likely to be older than those without [35, 283]; however, none of these studies has shown the difference to be statistically significant.

There are very few publications that have investigated the association between RPD and the various subtypes of newly presenting CNV [35, 55, 283]. Cohen et al reported a higher prevalence of RAPs in individuals with RPD (29.1%) than those without (7.8%) [35] although the authors specifically cautioned against the interpretation of this finding, given the small number of patients included in the study. This association was repeated when Ueda-arakawa demonstrated a prevalence of RPD of 83.3% in patients with newly presenting RAP. In contrast they also demonstrated RPD were rarely found in patients presenting with polypoidal choroidal vasculopathy. We found no association with RAP or other types of CNV in the present study. There may be several possible reasons for this. One potential explanation could be that our exclusion of images that were

deemed ungradable and/or eyes that had no appropriate early frames on the FFA could have had a large proportion of RAPs.

Another common finding from existing literature is that drusen and other features of ARM are usually present in eyes with RPD [30, 35, 284]. In our study conventional drusen ≥63µm were present within the ETDRS grid in 67.1% of all patients who presented with new onset CNV. In those in whom RPD were present the prevalence of drusen increased to 96.1%.

In keeping with Mimoun et al's first report, we find that RPD are best visualised using the red free and the blue channel of the FP when altered digitally with imaging software. Our detection rate with SD-OCT was significantly lower than that reported by others., with Ueda-Arakawa recently reporting a sensitivity of 94.6%. [201](NB-endnote error) Other groups have published rates that more inkeeping with ours, albeit be it in the contralateral eye of patients presenting with nAMD[259](Hogg et all-but endnote error). Possible explanations for this could include the larger field of the 35̊ FP when compared to the 6mm by 6mm field of the SD-OCT raster scan used in this study. Many of the central macula OCT sub-retinal drusenoiud deposits may have been obscured by any active CNV, rendering them visible only with a larger field FP.

The main limitation of this study is the unavailability of IR imaging. However, the combination of blue light and OCT would have mitigated against this. It is also possible that the CNV may have obscured some of the RPD.

In conclusion, the present study confirms findings from previous studies, that RPD have a high prevalence in eyes presenting with nAMD (22.1%), although at rates much lower than the prevalence of typical conventional drusen. The contralateral eyes with no nAMD have an even higher prevalence of RPD (31.5%) reflecting the difficulty in drusen grading in the presecnce of CNV. Most significantly, our study confirms that RPD are largely a bilateral finding and occur more frequently in females. Unlike other previous reports, we found no difference in their occurence between the different subtypes of CNV including RAPs.

Table 27: Comparison of characteristics in patients with or without RPD in the ipsilateral eye with newly presenting nAMD.

Data are n (%) ± SD

	Total n=231	**With RPD n=51 (22.1)**	**Without RPD n=180 (77.9)**	**P Value, RPD present versus absent**
Mean age (years)	80.4 ±7.6	80.09 ±8.21	80.49 ±8.22	
Right eye	113 (48.9)	27 (52.9)	86 (47.8)	0.530
Left eye	118 (51.1)	24 (47.1)	94 (52.2)	
Male	100 (43.3)	14 (27.5)	86 (47.78)	0.011
Female	131 (56.7)	37 (72.5)	94 (52.22)	

Table 28: FFA subtypes of nAMD with and without RPD.

Data are n (%).

	Total (n=231)	With RPD (n=51)	Without RPD (N=180)	P Value
Classic	27	6 (11.8)	21 (11.7)	0.933
Predominantly classic	16	4 (7.8)	12 (6.7)	
Minimally classic	50	12 (23.5)	38 (21.1)	
Occult	129	28 (54.9)	101 (55.6)	
PPCNV membrane	9	1 (2.0)	8 (4.4)	

Table 29: Contingency table for RAP with and without RPD.

	Total (n=231)	With RPD	Without RPD	P Value
RAP	9	1	8	0.688
No RAP	222	50	172	

Figure 20: A FP demonstrating RPD. There are multiple round and oval yellow-white lesions that join to form an ill -defined network of broad, interlacing ribbons.

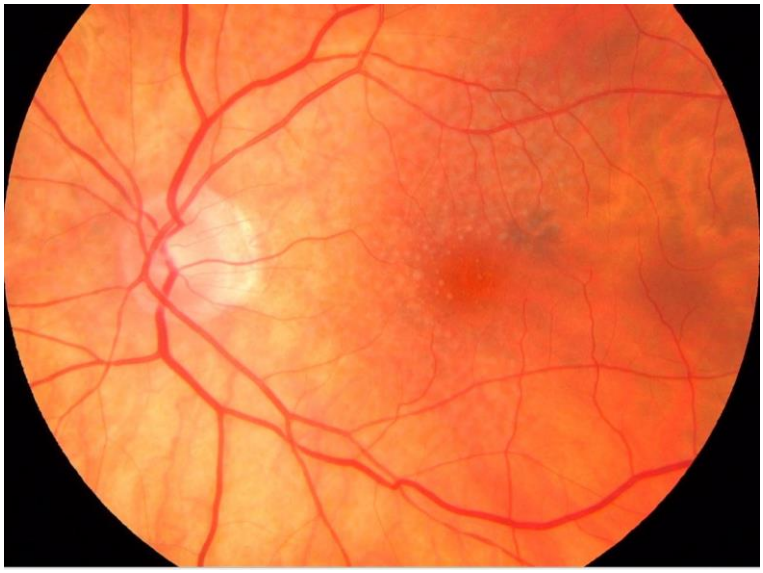


Figure 21: Enhanced visualization of RPD with digital enhancement of photograph to red free.

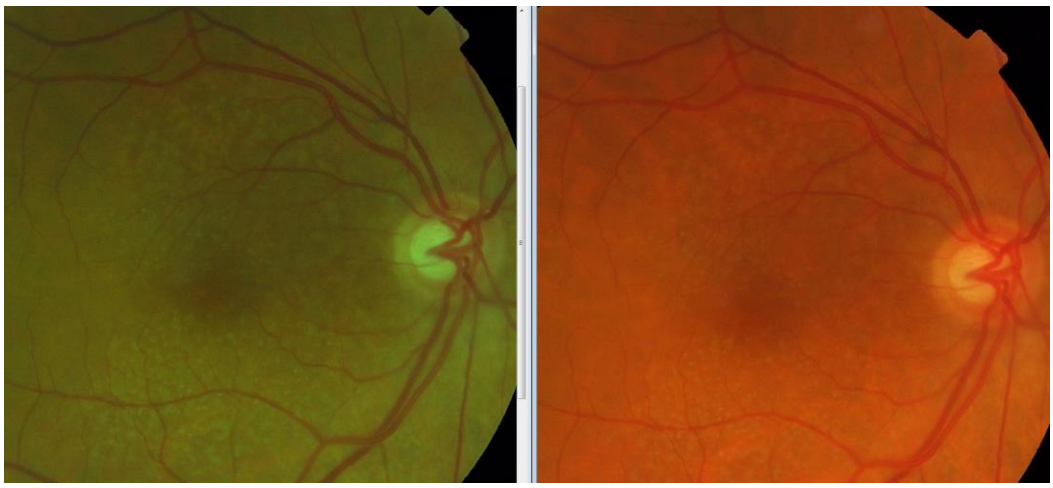
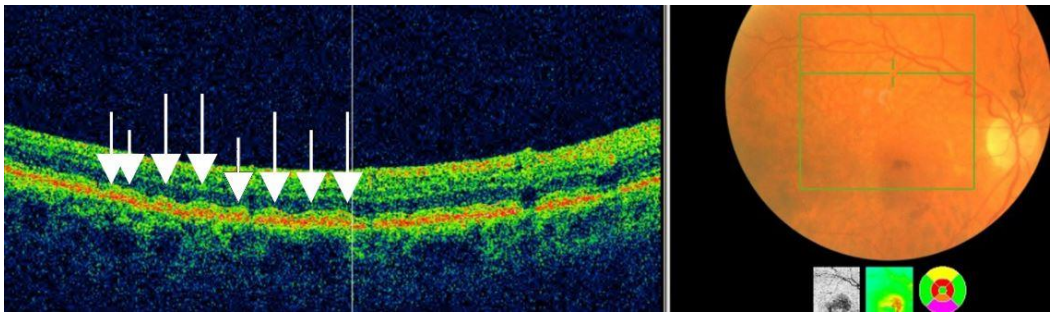


Figure 22: SD-OCT demonstrating discrete hyper-reflective collections in the subretinal space giving a saw-tooth appearance.



Chapter 11: General Discussions and Conclusions

11.1 ARM and AMD Prevalence in the UK

Although there are several publications on the prevalence of AMD in the UK, there remains a paucity of data from population studies from this country. This study provides the most detailed exploration of the epidemiology of AMD within a UK community based population to date. Chapter 3 provides contemporary prevalence rates for different stages of AMD in a UK population, and indicates the prevalence of advanced AMD is more common than previously thought (4.3%), reaching a maximum of 21.2% for the over 90 year age group. Despite this high prevalence of advanced AMD, approximately 50% of the over 65-year old population have no or minimal signs of ARM. This study confirms that persons with the earlier and intermediate stages of AMD are asymptomatic.

The prevalence of GA is more common than that of nAMD, with GA being 1.7 and 1.6 times more prevalent in the right and left eyes respectively. This difference remains when analysed for the worse eye, with GA still being 1.3 times more common than nAMD.

For the first time in a large population based study, this thesis reports self-satisfaction with participant's vision and association with AMD severity/grade. It shows that a large proportion of individuals (61.0%) with GA in their worse eye still remain satisfied with their vision. When AMD grade in the worse eye was the variable, nAMD is the only stage at which the majority of participants are

dissatisfied (59.4%) with the level of their vision. When the better eye was evaluated, there was a significant increase in subject dissatisfaction with vision, particularly for individuals with bilateral nAMD, with almost 90% of subjects being dissatisfied with the quality of their vision.

The study adhered to stringent methodology and has several strengths. It is the largest UK population based study to date to utilise mydriatic FP and internationally recognised grading standards. The study is likely representative of the Bridlington population, in that those recruited had similar demographics to non-attenders. Photographic standards were excellent, with 98% of attenders having a gradable photograph in at least one eye. Grading was performed in accordance with recognised standards established classification systems. Quality assurance processes were stringently adhered to. Secondary grading of 1 in 10 right eye images by an internationally recognised reading center confirmed the accuracy of the grading process, with substantial inter-observer variability. The almost identical grades of ARM/AMD in both right and left eyes, also reflects the accuracy of the grading.

11.2 Prevalence and characteristics of GA in the UK.

To the best of the author's knowledge, no detailed studies relating to GA are available from the UK population. Chapter 4 describes in detail the features of GA within this UK population. The information will be useful in the design of future clinical trials that evaluate the development or progression of GA or its treatment, as well as modelling the impact of GA on sight loss in the UK. In summary, GA has a prevalence of 2.5% for the worse eye in the over 65 year

Bridlington population, increasing with age to a maximum of 9.84% for those aged 85 to 90 years. Bilateral GA is not an infrequent finding, occurring in 34% of subjects with known GA. In the mainstay it is largely an eccentric condition, with only 31.5% of involved eyes having subfoveal GA. The majority of GA appears to occur in the perifoveal region, particularly GA that is associated with conventional drusen. Only a minority eyes with GA in the community have large areas of atrophy over 17.5mm^2; the mean area of GA was 4.51mm^2.

This thesis, in chapter 4, demonstrates there is a clear association between GA phenotype and the phenotype of drusen identified within the remaining fundus. In chapter 4 it is demonstrated that RPD are, in the mainstay, associated with horseshoe shaped GA, and have a high prevalence of 55.9% within this group. This is a previously unreported finding. The prevalence of RPD in eyes with round GA is considerably less (8.3%). There is a suggestion that GA in eyes with RPD may have larger areas of atrophy and are less likely to have foveal involvement. However, the mean VA remains the same secondary to a diffuse, central retinal pigment epitheliopathy that appears to be associated with RPD horseshoe shaped GA. This again, is a previously unreported finding. Unlike in the previous hospital based literature, we demonstrate most multifocal GA is still associated with conventional drusen.

11.3 Reticular pseudodrusen

In chapter 5, the prevalence of RPD in the over 65 year Bridlington population is estimated as 5.06% for either eye. This is the highest population based prevalence of RPD reported to date, and represents the only detailed report of

the epidemiology of RPD within a UK population. Out of all the population based prevalence studies performed to date, the BEAP Study is the only one to utilise and analyse the red-free channel of the FP, which enhanced RPD detection within the study by 13%. As such, this study possibly represents the most accurate measure of RPD prevalence to date. In the present study, the prevalence of RPD increases significantly with age, reaching a maximum of 27% in the over 90-year age group.

The BEAP study confirms that RPD occur most frequently in the upper outer subfield. Unlike previously reported, however, RPD do occur within the central subfield, albeit in a form that differs slightly in its appearance, and significantly reduced size.

The BEAP study confirms that RPD have a female preponderance, with a higher gender specific prevalence rate in women. They are commonly found in association with other signs of ARM, including drusen over 125µm (50 % of the time) and pigmentary changes. Isolated RPD, in the absence of conventional drusen, is an uncommon finding but does occur. Approximately 1 in 4 subjects with advanced AMD will have evidence of RPD in either eye. This thesis also reports, for the first time, the prevalence of RPD in eyes with PPCNVs. There is a suggestion that RPD are associated with visual dissatisfaction, and this may be associated with a central pigmentary epitheliopathy that is sometimes seen within RPD eyes.

To the best of the author's knowledge, to date there is no reported breakdown of RPD prevalence into subtypes based on their fundus features from within a community based population study. This is in part explained by the lack of consensus regarding RPD sub-classification. The present population based study confirms that dot RPD are a frequent finding, identified in 54.8% of eyes with RPD. The lower prevalence in the present study may, however, reflect the different study population or the different diagnostic imaging tools utilised in the different studies. In the BEAP, only FP with digital enhancement was used. Previous publications report that dot RPD were most commonly identified using IR, and ribbon RPD were more detectable using FP. As the present study used FP, ribbon RPD were the most frequently identified subtype. This study, further demonstrates that the different subtypes of RPD can occur together or independent of each other. It is, therefore, reasonable to assume that one type is not a more advanced form of RPD development. It remains to be determined if dot or ribbon RPD confer different risks for the development of either GA or nAMD.

11.4 Peripapillary choroidal neovascular membranes.

Chapter 6 reports several new findings, including the prevalence of PPCNV within an elderly Caucasian population in the UK. To the best of the author's knowledge, this represents the first study that has specifically reported the population prevalence of PPCNV, as all previous publications on the subject have arisen from hospital based populations, and therefore included predominantly symptomatic individuals, and therefore carried inherent

selection bias. PPCNVs (grade 4c AMD) were an infrequent finding, with a prevalence of 0.29% for individuals over 65-years of age. This is considerable lower than nAMD (grade 4b AMD), which within the same population had a prevalence of 1.8% for the worse eye. There was a female preponderance, with 70% of PPCNVs occurring in females. This difference was maintained in gender specific prevalence rates of 0.36% and 0.19% for females and males respectively.

Previous publications have reported that PPCNVs accounted for less than 10% of all CNVM. This figure is confirmed in the BEAP, in which PPCNV accounted for 12 out of a total of 90 cases of CNVMs, representing 13.3% of all prevalent CNVs identified. This study reports that the majority of cases of PPCNV are unilateral.

There is a myriad of published associations between PPCNV and other conditions. These are mainly single case reports or small case series. In addition, the larger hospital based studies may also give a poor representation of the true associations between PPCNV and other conditions as small, nasal or age-related membranes may remain asymptomatic. In Chapter 6, 90% of eyes with PPCNV had evidence of drusen ≥63µm within the macula, an association which is far higher than previously published. Furthermore, in the current series, 30% of PPCNV were associated with RPD, a finding which had previously been unreported. Previous studies on PPCNV's have graded the macula for age-related change, but not reported or graded the peripapillary area for degenerative changes. In this report, these features have been investigated for the first time, and find that all (100%) of our subjects had RPE pigmentary

changes within half a disc diameter of the disc margin (in both affected and contralateral eyes). As drusen and pigmentary changes within the macula are the known hallmarks of both GA and CNV, it seems logical to consider these changes in the peripapillary area as potentially pathological for PPCNV and for them not to be overlooked.

The association of PPCNV with angioid streaks is well established, but in chapter 6 it is reported that in 10% of subjects with PPCNV, there were identifiable angioid streaks. This is a more frequent association than previously published (although the numbers are small), and highlights an overlap between the aetiologies of PPCNV in patients with angioid streaks within the elderly, and suggests a possible tendency for an association with small membranes that remain asymptomatic. In the present study of asymptomatic individuals, a large proportion of PPCNVs (42%) were nasal to the disc margin and no individual in this series was thought to have developed direct visual loss from PPCNV. This finding is very distinct from that in some previous hospital studies of symptomatic patients. This study suggests, by inference that up to two thirds of PPCVN may remain asymptomatic.

11.5 Diagnostic accuracy of SD-OCT for diagnosing nAMD

In chapter 7, SD-OCT in comparison to the reference standard of non-stereoscopic FFA is shown to be highly sensitive (100%) at detecting newly presenting nAMD in the setting of a specialist AMD clinic, where the investigations are interpreted by trained specialists. The specificity of SD-OCT alone for detecting CNV was 80%. The study highlights a significant number of

false positive cases identified with the use of SD-OCT alone: a rate of 16.9%, and confirms that FFA is still the diagnostic gold standard for diagnosing nAMD. This is the first report to compare non-stereoscopic FFA, which offers a real world comparison between the two imaging modalities. This chapter highlights possible limitations of SD-OCT for the imaging of nAMD such as the small field (6mm by 6mm) used for routine scans of the macular area, where eccentric pathology and peripapillary lesions could be missed but that would otherwise be detected with standard protocols within the larger 35° field of FFA. The chapter demonstrates that the high false positive rate represents difficulty in correctly identifying areas of hyperreflectivity on SD-OCT that represent active CNV, and distinguishing them from those that represent inactive gliosis, particularly in the setting of chronic lesions. SD-OCT, not unexpectedly, seems to allow easy identification of structural changes that indicate there has been a previous or currently active CNV using SD-OCT. However, it is unable to determine whether the fluid detected is from an active CNV at diagnosis. Furthermore, other causes of intraretinal cysts or fluid do occur and may confound diagnosis of CNV based purely on the presence of such spaces.

11.6 Causes of false positive diagnosis with SD-OCT for nAMD

In chapter 8 the reasons behind the false positive diagnoses identified from chapter 7 were explored. There is clearly a sparsity of published data on the topic, with no dedicated publication covering the topic in detail and the chapter may be of clinical importance, in that it discusses potential diagnostic pitfalls of SD-OCT in AMD. The chapter highlights some of the major problems with SD-

OCT for diagnosing nAMD, where 31.8% of false positives occurred secondary to non-drusenoid PED's. Some non-drusenoid PEDs in the series most likely represent regressing drusenoid PED's before the RPE flattens in the progression to frank GA, and highlights the importance that not all PED's will correspond to an occult lesion with FFA. It reminds the clinician that drusen are a dynamic structure, and can have various appearances depending on their stage of regression.

RPE elevation and cyst-like spaces occurred in areas of GA or peri-lesional areas. It appears that on SD-OCT these can sometimes be confused for nAMD. Small areas of presumed SRF or IRF on SD-OCT clearly pose a potential for diagnostic errors and confusion with nAMD, in the absence of other ocular pathologies to account for these changes. In addition, the findings from this study suggest that RPE elevation may be a precursor to GA progression or development over time. The chapter highlights the fact that GA is a heterogeneous condition and the current classification systems fall short in recording and recognising all its features particularly as it often develops as a continuum from regressing drusenoid PEDs. A newer multimodal classification system is likely required.

An unexpected and important finding within chapter 8 is that 57% of SD-OCT false positive eyes were treated for nAMD, despite the absence of angiographic features of active CNV when reviewed under study conditions. The implication of this finding is that FFA interpretation within this cohort is associated with a high degree of diagnostic error. A likely explanation is that most false positive eyes (87.2%) had significant pigmentary changes being evident on FP that would

have confounded the appropriate interpretation and resulted in misclassification. The SD-OCT appearance may also have introduced an element of bias.

In chapter 9 it is confirmed that RPD are not a finding restricted to eyes with AMD but rather common among diseases where the pathophysiological mechanisms primarily involve damage to the BM and the RPE, whether genetic in origin or degenerative. The prevalence rates of RPD in eyes with angioid streaks (36.5%), and for the first time the prevalence of RPD in eyes with newly presenting AFVD is reported. The study supports the concept that RPD appear to occur with high but variable frequencies in eyes with various retinal and macular pathologies.

11.7 Prevalence of RPD in newly presenting nAMD

Chapter 10 confirms findings from previous studies that RPD have a high prevalence in eyes presenting with nAMD (22.1%), although at rates much lower than the prevalence of typical conventional drusen. The contralateral eyes with no nAMD had an even higher prevalence of RPD (31.5%) reflecting the difficulty in drusen grading in the presence of CNV. Most significantly, the study confirmed that RPD are largely a bilateral finding and occur more frequently in females. Unlike other previous reports, no differences in the occurrence of RPD amongst the different subtypes of CNV (including RAPs) were found.

In conclusion, this study represents the largest epidemiological investigation of AMD within a UK population to date. Some new findings that will hopefully be

of clinical or academic use to both ophthalmologists and public health specialists in the future are included in this thesis.

11.8 Future Directions

A major limitation of this research is that it is confined to a population that is 100% Caucasian. It therefore provides no information on the non-Caucasian UK population, making its generalization within the UK multiethnic population difficult. A further population based UK multiethnic study would be appropriate to explore whether prevalence rates differ between different ethnic communities living within the UK. The restriction of the study to include only Caucasian subjects may also be an advantage, as a larger population may have been required to provide acceptable confidence limits. However, studies in other UK areas would be required to provide data on AMD prevalence across all populations. Further limitations include the exclusion of individuals with known visual impairment, which will have resulted in an underestimation of true AMD prevalence. Also, stereoscopic images would have been a preferred imaging modality.

No population based study to date has reported prevalence with multimodal imaging, but rather FP alone. Future prevalence studies would benefit from the use of SD-OCT, and multimodal imaging which would allow better classification of GA, ACA, outer retinal atrophy and RPD. An OCT evaluation of the choroid in this population would have added further understanding of the different AMD features described.

There are currently no figures for the incidence of AMD (different types) in the UK. The Bridlington study would serve as good cohort to determine 10 year incidence rates for the different AMD subtypes in a Caucasian UK population.

Limited data exists on the community prevalence of ACA and its association with RPD and AMD. This would be a useful addition to AMD literature and could be addressed in future epidemiological studies using enhanced depth OCT imaging of the choroid.

In this thesis I have demonstrated the association with AFVD and RPD. It remains to be determined whether those with RPD have a less favourable prognosis in terms of incidence of GA and formation of CNVM, than those without. Further work is required to explore this.

I intend on using the BEAP database, which includes driving status and visual acuity to explore the epidemiology of drivers within an elderly UK population. Older adults are the fastest growing age group and they tend to continue to drive into late life. Older people have higher crash rates per mile driven. Accidents involving the elderly are more likely to be severe and involve a fatality. Some studies suggest these higher crash rates are in part due to declining vision as well as many other factors. Older adults may place self-restrictions on their driving such as driving fewer miles and only in good weather in the daytime. Some may stop driving altogether. In the UK visual driving standard is tested initially on application for a driving license. You must be able to read (with glasses or contact lenses, if necessary) a car number plate

made after 1 September 2001 from 20 metres. You must also meet the minimum eyesight standard for driving by having a visual acuity of at least decimal 0.5 (6/12) using both eyes together as measured in the Snellen chart.

I intend on using the BEAP data to explore driving habits in an ageing UK population and any association with VA reduction. I will estimate the number of individuals driving with reduced VA. I will establish how many people have reduced vision and may not meet the legal driving VA minima but would potentially meet it with new spectacles of if they were to have their cataracts removed.

Chapter 12: Appendix 1 STROBE Statement

	Item No	Recommendation
Title and abstract	1	*(*a) Indicate the study's design with a commonly used term in the title or the abstract: Prevalence of Age-Related Macular Degeneration in an Elderly UK Caucasian Population - The Bridlington Eye Assessment Project (BEAP): A Cross Sectional Study.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Importance: There is paucity of data on prevalence and asymmetry of age-related macular degeneration (AMD) in the UK population. Objective and Purpose: To determine AMD

prevalence in an elderly Caucasian UK population.
Design: Cross-sectional population study, 2002-6.
Participants: Residents in the study area of Bridlington aged 65 years or older.
Methods: Full ophthalmic examination was undertaken in 3549 participants, of eligible 6319 Caucasian population (response rate of 56%). Colour fundus photographs were graded masked using the Rotterdam Classification for 3475 (98%) participants with gradable images. Prevalence for different AMD grades were calculated.
Results: AMD prevalence in the worst eye were 38.5 % grade 0, 41.4% grade 1, 12.8% grade 2, 2.8% grade 3, and 4.6% grade 4. Geographic atrophy(grade 4a) occurred in 2.5%, and neovascular AMD (grade 4b) in 1.8%. Prevalence increased with age such that grade 4 (advanced) AMD was 2.2% in the 65-69 years group, 15.8% for the 85-90, and 21.2% for over 90 years. There was significant asymmetry between the 2 eyes of individuals with advanced AMD ($p<0.001$), such that vision loss was unilateral. Persons with more advanced AMD grades were more likely to be dissatisfied with their vision.
Conclusions: Advanced AMD occurs more commonly in the UK Caucasian population than previously reported. Significant asymmetry between the 2 eyes occurs in individuals with unilateral advanced AMD so that visual impairment statistics do not represent true prevalence of advanced AMD. Persons with more advanced AMD are more likely to be dissatisfied with their vision.

Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported There is paucity of data on prevalence and of age-related macular degeneration (AMD) in the UK population. Many previous studies are old, use non-standard or old classification systems and are often small with less than 1000 subjects. No study has reported the prevalence of RPD or PPCNV in the UK
Objectives	3	State specific objectives, including any pre-specified hypotheses 1. To determine the prevalence of different stages of ARM/AMD in the elderly UK population and their correlation with measured visual acuity and

		explore associations with systemic risk factors. 2. To report the community prevalence and risk factors of reticular pseudodrusen (RPD) and to determine their morphology and retinal distribution. 3. To provide a measure of prevalence of asymptomatic PPCNV. 4 To provide a detailed measure of the prevalence of GA within the UK and provide information regarding the features of GA including mean area, the prevalence of multifocal GA and proportion of eyes with foveal involvement.
Methods		
Study design	4	Present key elements of study design early in the paper Cross sectional population based
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Bridlington, East Yorkshire, UK. Recruitment and data collection from 2002-2006
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of selection of participants All Bridlington residents aged aged 65 years and over and registered with a GP. Exclusion criteria: All subjects known to be moving in/out of the area, bed bound individuals, patients with known dementia and those registered blind/partially

		sighted.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable FP of subjects are graded for ARM/AMD and a Modified Rotterdam Grade assigned. FP grading according to the International Classification System of AMD. Structured interview with research nurse as described in more detail in thesis, collecting medical history and demographic patient data.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Modified Rotterdam grade of AMD for each eye and worse eye of each subject. Area of and characteristics GA, RPD and PPCNV.
Bias	9	Describe any efforts to address potential sources of bias Secondary grading by CARF Cross sectional population based study
Study size	10	Explain how the study size was arrived at Population based study
Quantitative variables	11	Explain how quantitative variables were handled

		in the analyses. If applicable, describe which groupings were chosen and why Logistic regression with associated conditions that are also age-related such as cataracts.
Statistical methods	12	*(a)* Describe all statistical methods, including those used to control for confounding Statistical analysis was performed using Stata 12.0 (StataCorp, College Station, TX) and SPSS v.22 (IBM Corp. Armonk, NY)
		(*b*) Describe any methods used to examine subgroups and interactions Logistical regression anaylsis
		(*c*) Explain how missing data were addressed Subjects with no available photograph were excluded from analysis
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed 3549 individuals participated in the initial examination, corresponding to 56% of subjects

		within the eligible study population. A total of 3475 participants had gradable photographs in at least one eye with 226 having ungradable images in at least one eye. 74 individuals had ungradable images in both eyes.
		(b) Give reasons for non-participation at each stage Lack of consent or response to recruitment letter. Cataracts were responsible for reduction in FP clarity making images un-gradable.
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders The mean age of individuals with gradable photographs was 75.0 years (SD 5.9, range 65-100, 95% CI 74.8 - 75.2). Mean Jarman score for participants was 1.75.
		(b) Indicate number of participants with missing data for each variable of interest Individuals with ungradable photographs tended to be older, with a mean age of 77.7 years (SD 6.0, range 66-95, 95% CI 77-78, p<0.001 Mann Whitney U Test). There was a slightly increased female preponderance (58:42) in the ungraded

		group but this difference did not reach conventional levels of statistical significance ($p=0.46$, Chi^2).
Outcome data	15*	Report numbers of outcome events or summary measures-see results
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included-see results
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period-NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives-See thesis • Grade 4a AMD (GA) occurred in 2.5%, neovascular AMD (grade 4b) in 1.8%, and peripapillary CNV (grade 4c) in 0.3% in the UK population. • Prevalence increased with age such that

		grade 4 AMD increased from 2.2% in the 65-69 years group to 15.8% for the 85-90, and 21.2% for participants over 90 years. • Advanced AMD was more 4 times more likely to be associated with visual dis-satisfication that lower grades.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias-see thesis Caucasian only population FP only with absence of multimodal imaging, particularly SD-OCT, which limits the grading of RPD. Exclusion of blind/partial sighted subjects
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence-see thesis
Generalisability	21	Discuss the generalisability (external validity) of the study results-see Chapter 3 discussion Generarlisable to an elderly Caucasian population in the UK
Other information		

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based- The Bridlington Eye Assessment Project was funded by an unrestricted grant from Pfizer. We would also like to thank the following organisations for financial support of the Project: Pharmacia, Yorkshire Wolds and Coast Primary Care Trust, The Lords Feoffees of Bridlington, Bridlington Hospital League of Friends, The Hull and East Riding Charitable Trust, The National Eye Research Centre (Yorkshire), The Rotary Club of Bridlington, The Alexander Pigott Wernher Memorial Trust, Bridlington Lions Club, The Inner Wheel Club of Bridlington, Guide Dogs for the Blind, Soroptimist International of Bridlington, and The Patricia and Donald Shepherd Charitable Trust.

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