Early **D**etection of **N**eovascular **A**ge-related macular degeneration (AMD)

Statistical Analysis Plan Version 1 21/01/2020

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Approved by: [redacted]

J.Cook 21/1/20

Statistician

U.Chakravarthy [redacted] 21/1/20

Chief Investigator

Introduction

Background and rationale

Neovascular age-related macular degeneration (nAMD) causes severe visual loss and is the most common cause of blindness in persons > 50 years old in the western world (Royal College of Ophthalmologists guidelines 2009). In recent years, there have been major advances in the clinical management of patients with nAMD, notably the introduction of anti VEGF treatments. When active nAMD is confirmed, treatment with anti VEGF therapy is initiated (Chakravarthy 2010, IVAN investigators 2012; Martin 2012). In the early phases of treatment (i.e. up to about one year), at each subsequent visit which is usually on an 8 week cycle, patients are re-assessed to evaluate disease activity. Thus there is an opportunity to obtain information on unaffected fellow eyes of patients with nAMD in one eye.

Approximately 8-10% of patients with nAMD in one eye will develop the same condition in the fellow eye per year. Detection of nAMD at a stage when damage to the retina is not permanent with prompt initiation of treatment could result in much better preservation of sight. Therefore there is a clear need for an easily and rapidly performed cost effective monitoring test that will detect the onset of nAMD with high diagnostic accuracy.

Managing neovascular AMD presents an enormous burden to the NHS. Ophthalmology accounts for 10% (five million per year) of all outpatient attendances to the NHS, and age-related macular degeneration accounts for 15% of all ophthalmology outpatient attendances. (Age-related macular degeneration guidelines 2009 Royal College of Ophthalmologists).

Scrutiny of the outcomes from the large clinical trials shows that if treatment is commenced when acuity is better than 73 letters (Snellen equivalent 6/12), over 90% maintain this level of vision or better (Martin 2012, IVAN 2012). Better acuity is associated with smaller nAMD lesions and thus early detection of nAMD and prompt initiation of treatment will result in final visual outcomes that are consistent with good visual function. The proposed research is particularly important because (1) there is a large patient pool whose care pathway requires regular visits and monitoring (every 8 weeks) offering the ideal situation for a study of early detection of nAMD in fellow eyes of patients with nAMD in one eye (2) these patients are subjected to tests of function (acuity) and tomography and it is current clinical practice to acquire information on both eyes at every visit (3) the tomographic examination is quick (performed without the need for pupillary dilation and the quality of the tomograms are high as all the NHS units offering anti VEGF therapies have invested in high resolution Fourier domain OCT technology (4) the patients are motivated and the NOD has shown that attendance is high with dropout less than 10% per annum.

Interventions

The interventions (index tests) to be evaluated are:

1. Fundus evaluation: a positive fundus evaluation is one as determined by an expert showing signs of nAMD on the fundus.
2. Visual acuity using the early treatment diabetic retinopathy study chart (ETDRS): a positive test is one where there is a reduction of 10 letters or more in best corrected visual acuity (BCVA).
3. Amsler test: a positive Amsler test is, as assessed by the clinician, appearance of a new area of distortion or blank spots when previously there was none or clear evidence of increase in the area of distortion or scotoma.
4. Optical coherence tomography (OCT): a positive test is abnormal findings indicative of nAMD as interpreted by an experienced ophthalmologist, specifically it is a positive answer to any of the following: SRF on OCT, neovascular PED on OCT, IRF on OCT or any other reason for OCT being positive.
5. Patient's subjective assessment of vision: the patient is asked "how is your vision in the unaffected eye?" The patient is prompted to answer one of the following four possibilities "about the same or better", "a bit worse", "worse" or "much worse". A positive test is one where the patient reports "much worse".

Aim and objectives

Aim: To identify the optimum non-invasive test strategy that will robustly detect nAMD in fellow eyes during follow-up in secondary care of persons with nAMD in the first affected eye.

Objectives

Primary objective: determine the diagnostic monitoring performance of the interventions (ETDRS visual acuity; fundus evaluation of signs of nAMD; the Amsler test; clinical assessment of images captured by OCT; patient's subjective assessment of vision against the reference standard of fundus fluorescein angiography);

Secondary objectives:

1. Develop an economic model to identify an optimal monitoring regime;
2. Develop a risk prediction model using baseline characteristics to predict the development of nAMD in the study eye:
3. Create a cohort (including a Bio bank) which can be used for future prognostic and diagnostic studies.

Design

The study design is a multi-centre prospective cohort diagnostic accuracy study with 3 year follow-up. Once enrolled into the study, the participants will be monitored following standard clinical practice in the diseased eye. The standard of care in the NHS for patients newly diagnosed with nAMD is regular (approximately every 8 weeks) assessment and treatment if required. At each monitoring visit, patients will be examined using all index tests in the study eye (unaffected) and a reference standard measurement triggered if any of the index tests are positive. All patients will be followed-up according to standard clinical practice until confirmed treatment for nAMD in the study eye or until 3 years from enrolment, whichever is sooner. Patients who do not have confirmed nAMD in the study eye during the follow-up period will have a FFA at 18 months and exit. The study has been designed to have minimum impact on the current patient care pathway.

Statistical Principles

Sample size

The sample size is based upon comparative diagnostic accuracy to ensure the ability to detect differences in sensitivity and specificity between candidate tests. The calculation is based upon McNemar's test. (Obuchowski 1998) Under the primary analysis, a positive candidate test result will be defined as any positive result during the monitoring period on the respective test. At 2-sided 5% significance level and 90% power, a paired difference of 15% (80% to 65%) in sensitivity will require 491 participants (560 allowing for indeterminate/missing data results - including patients lost to follow-up cumulatively of up to 12%) given a cumulative incidence of 28% at 3 years. (Karnon 2008) This calculation assumes a disagreement between tests of 0.30 which was based upon data from a diagnostic study involving OCT for diagnosis glaucoma (HTA reference 09/22/111). A smaller difference in specificity will be identifiable (7%; 94% to 87% with power and significance levels as before) given most participants will not convert during the 3-year follow-up period even if the maximum level of disagreement occurs. The reference sensitivity and specificity values used in this calculation are the values observed for OCT in a pilot study with a similar study design. (Parnick-Silver 2012) Differences in sensitivity and specificity of at least 20% will also be detected at the same power and significance levels even if the sensitivities/specificity are substantially lower (e.g. 60 to 40%) or the level of missing data is higher (e.g. 20%). These calculations conservatively assume maximum possible disagreement between tests. A sample of this size would be of sufficient size for other measures of diagnostic performance (e.g. the sensitivity and specificity of individual technologies will be estimated to 95% confidence interval of width 16% and 10% respectively given a sensitivity/specificity of 65% or higher). Such a sample will also provide a sufficient sample for the GEE analysis given the anticipated gain in precision due to use of multiple repeated measures over

time. (Rochon 1998) Similarly, this sample will be more than sufficient for the development of a risk prediction model with over 130 events (conversions to AMD) anticipated and given 10 events per predictor variable/contract are typically recommended. (Peducci 1995).

Interim analyses

No formal interim analyses are planned. The TSC will monitor event (nAMD conversion) data and also the disagreement between tests (blinded to individual test results and differences) to evaluate the key assumption in the sample size calculation. A single final set of analyses is planned once the study has recruited and data has matured.

Time points of outcome collection

In order to assess the sensitivity and specificity of the diagnostic tests under evaluation, each of the diagnostic tests being assessed will be performed on the study eye at each routine clinic visit during the period of follow up.

Results from the diagnostic tests according to the definitions will be recorded on a standardised case report form. If any diagnostic tests are positive this will trigger the request for an FFA and the absence/presence of nAMD (and classification) will be recorded. These data will be uploaded to the study website by study staff.

Table 1 Timing of outcome collection

Assessment	Recruitment	Routine visits (approx. every 8 weeks)	If any diagnostic test positive	If FFA positive	18 months	exit
Assessment of eligibility criteria	✓					
Written informed consent	✓					
Baseline vision and risk factors	✓					
Blood collection	✓			✓		✓
Diagnostic tests: • Subjective patient vision • AMSLER • Visual acuity • OCT • Fundus examination • Autofluorescence (only if available)	✓	✓			✓	✓
Reference standard: FFA	✓		✓		✓	✓
Post conversion case note review						✓
Upload of required imaging to reading centre for analysis	✓		✓		✓	✓

Eligibility

Patients with newly diagnosed nAMD in one eye and an unaffected second eye (study eye).

Inclusion criteria:

- Newly diagnosed nAMD in one eye and an unaffected second eye (diagnostic FFA to be within 6 weeks prior to consent)
- About to commence or recently commenced anti VEGF therapy in the first eye
- Age 50 -95

Exclusion criteria:

- patients with a history of nAMD in both eyes;
- nAMD in study eye detected at baseline;
- presenting visual acuity worse than 68 letters;
- retinal pathology in the study eye which can confound subsequent assessments (e.g. diabetic retinopathy, macular hole);
- not undergoing regular monitoring in standard of care;
- patients who cannot give informed consent;
- unable to undergo a fundus fluorescein angiography (FFA) test;
- patients whose baseline FFA was more than 6 weeks ago

Change of status

Participants will remain in the study unless they choose to withdraw consent or if they are unable to continue for a clinical reason. All changes in status with the exception of complete withdrawal of consent will mean the participant is still followed up for all study outcomes wherever possible. All data collected up to the point of complete withdrawal will be retained and used in the analysis unless the participant requests this to be destroyed and excluded. If the participant had previously consented to and donated blood for storage, and the participant later withdraws consent, they may also request for their donated blood to be destroyed.

Baseline patient characteristics

Table 1 of the dummy tables outlines the baseline characteristics which will be collected and summarised.

Outcomes

Primary outcome measure

The **primary diagnostic performance outcomes** will be the sensitivity and specificity of the index tests on detection of nAMD in the study eye in a monitoring setting.

The **primary economic outcome** will be the incremental costs (to the health service) per quality adjusted life year (QALY) gained.

Secondary outcome measure

Secondary diagnostic performance outcomes will include diagnostic odds ratio, likelihood ratio, and proportion of indeterminate tests. The performance of combinations of tests will be evaluated.

Other outcomes: time gain of early detection, visual acuity at diagnosis, performance of a risk predictor algorithm according to baseline characteristics, the establishment of a well characterised cohort of clinical and biological data for future research.

Analysis

- **Analysis objectives**

1. Determine the diagnostic monitoring performance of the interventions (ETDRS visual acuity; fundus evaluation of signs of nAMD; the Amsler test; clinical assessment of images captured by OCT; patient's subjective assessment of vision) against the reference standard of fundus fluorescein angiography.

2. Develop a risk prediction model using baseline characteristics to predict the development of nAMD in the study eye
3. Quantify the time to diagnosis and risk of conversion
4. Explore the impact of CNV subtype on time to conversion

- **Method**

Participants are categorised as nAMD or not nAMD according to the presence of a positive FFA result (as assessed by the responsible ophthalmologist) during the follow-up period. To address the analysis objectives, the following analyses are planned:

Analysis Objective 1: Determine the diagnostic monitoring performance of the interventions

Analysis A. Person level diagnostic accuracy analysis.

Table 3 outlines the different definitions for the reference standard and an index test positive result for all the analysis under the person level diagnostic accuracy analysis.

Under the main analysis approach (analysis A1, see **table 3**), repeated monitoring test assessments are collapsed over time to give a single candidate test result (positive or negative). This is compared with a single final local FFA result from the participant. Other approaches will be considered by varying the definitions for reference standard and index tests included in the analysis (analysis A2 – A7, see **table 3**).

Participant inclusion criteria for analysis A

For each of the 5 index tests, the following criteria will be applied separately:

1. Last FFA result shows no nAMD:

The participant will be included if there is **at least one prior** index test result during the follow-up period.

2. Last FFA result shows nAMD:

The participant will be included if there is an index test result within the previous 3-months. If a participant does not have an index test result within the previous 3-months of their last FFA the FFA will not be considered valid and we will look for an earlier FFA result for the participant and apply rules 1 and 2 again on the earlier FFA.

If no follow-up FFA is available which satisfies either criteria, then the participant will be excluded. If no follow-up FFA is available, the participant will be excluded from the analysis.

Definition of valid follow-up period

If a participant is included as defined above, then the valid follow-up period will be from baseline until the date of the last valid FFA (as defined above).

Index test result definition for analysis A

For each index test, multiple test results will be collapsed into a single test result. Any positive test result over the valid follow-up period will be classed as an overall positive result. To be a negative index test result, all index test results must be negative. The classic 2x2 table for assessing diagnostic accuracy with our definitions is provided in Table 2 below.

Table 2 Two by two table: definitions of test and disease status.

	Index positive	Index negative
Reference standard positive: nAMD	**True positives** Any reference standard indicates nAMD in study eye AND Any index test result is positive during valid follow-up period	**False negatives** Any reference standard indicates nAMD in study eye AND Index test negative for all available prior time points
Reference standard negative: No nAMD	**False positives** All reference standard results indicate no nAMD in study eye AND Any positive index test result during valid follow-up period	**True negatives** All reference standard results indicate no nAMD in study eye AND Index test negative for all available prior time points

Sensitivity and specificity will be calculated with 95% confidence intervals calculated using the Agresti-Coull method (Zhou 2002). Positive and negative likelihood ratios will also be calculated with 95% confidence intervals calculated using the method in Zhou 2002. Diagnostic odds ratios and the proportion of indeterminate tests will be calculated with 95% confidence intervals. A ROC curve will be plotted and the area under the ROC curve calculated using the trapezoidal rule for visual acuity tests. The standard error for the AUC will be calculated using the method of DeLong, DeLong and Clarke-Pearson (1998) and used to form an asymptotic normal 95% confidence interval.

Monitoring sensitivity and specificity of the tests will be compared using McNemar's statistical test (with 95% confidence intervals produced using Newcombe's method) (Newcombe 1998).

For comparing sensitivities under the primary analysis, the McNemar 2x2 table will be constructed using only patients who have had a positive FFA result. They will be classified as having a positive index test, if the index test has any positive results during the valid follow-up period. They will be classified as having a negative index test, if all prior available tests have been negative during their valid follow-up period. For comparing specificities under the primary analysis, the McNemar 2x2 table will be constructed using only patients who have had negative FFA results throughout using the same index test classification.

Analysis A2 analysing the index tests collapses the index tests as in table 2 but only uses index tests from the individual's last 6 study months (not the entire study period). A positive index test result out-with the 6 months window therefore does not lead to a positive test result at the collapsed individual

level. The individual diagnostic performance of the tests are calculated using this index test definition and the McNemar comparisons of sensitivity and specificity as detailed above.

The third approach to the index tests uses the index test at the individual's last study visit only (Analysis A3). If a participant developed nAMD according to the reference standard during the follow-up period, only the index test data from their visit where nAMD was diagnosed will be used. If they did not develop nAMD during the follow-up period, index test data from the last visit for which their reference standard is available will be used.

Analyses A1, A2 and A3 use the local FFA as assessed by the responsible ophthalmologist as the reference standard. If a participant has a negative local FFA result but exits the study at that point because of a clinical diagnosis of nAMD by the ophthalmologist, then they will be classified as reference standard no nAMD.

Alternative person level diagnostic accuracy analyses are planned that will use two alternative reference standards:

- An alternative reference standard using the FFA result as determined by the reading centre (analysis A4 using all valid index test results, analysis A5 using index tests from the last 6 months and analysis A6 using the index test results from the last visit). Analyses A4, A5 and A6 comprise the individual diagnostic performances of the tests and the McNemar comparisons.
- An alternative clinical reference standard will also be used. A positive clinical reference standard result will be assigned if there is <u>either</u> a positive local FFA <u>or</u> a positive clinical diagnosis. A negative clinical reference standard result will be assigned if there is a negative FFA <u>or</u> an inconclusive local FFA and negative clinical diagnosis <u>or</u> FFA not done and a negative clinical diagnosis. Analysis A7 will use the clinical reference standard as defined above and all the valid index test results will be used. The individual diagnostic performances of the index tests and McNemar comparisons will be included.

A summary of the reference standard and index test definitions and the corresponding analyses is included below

<u>Reference standard definitions:</u>
A = Local FFA. Reference standard is positive if the local FFA indicates nAMD. Reference standard is negative if local FFA indicates no nAMD throughout.
B = Reading centre FFA. Reference standard is positive if the reading centre FFA indicates nAMD at any time point and negative if it indicates no nAMD throughout.
C = Clinical reference standard. Reference standard is positive if the local FFA indicates nAMD or a clinician diagnosis of nAMD at any time point. Reference standard is negative if the local FFA is negative/inconclusive/not done and the clinician diagnosis is negative.

Index test definitions:
A = Collapsed index test results from whole valid follow-up period. Positive if there is any positive index test and negative if the index test is negative throughout. All index tests during the valid follow-up period are used.
B = Collapsed definition from last 6 months of monitoring. Only uses index tests within 6 months of participant's study end date. Positive if there is any positive index test within the final 6 months and negative if the index test is negative throughout the final 6 months.
C = Index test result at participant's last available visit only (within a 3 month window of the last FFA).

Table 3 Description of the different reference standard and index test results definitions for analysis A

Analysis number	**Analysis**	**Reference standard definition of disease**	**Index test definitions**
A1 (Main analysis)	Primary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	A	A
A2	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	A	B
A3	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	A	C
A4	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	B	A
A5	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	B	B
A6	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	B	C
A7	Secondary diagnostic analysis-diagnostic performance of the individual tests. Paired comparison (McNemar) of sensitivity and specificity between the tests.	C	A

Analysis B. The secondary complex analysis which utilises repeated test results.

Analysis B will use reference standard definition A (local FFA result will be reference standard).

A GEE modelling approach will be used to allow the simultaneous modelling of sensitivity and specificity in a regression framework and use of multiple test results per participants over time. A GEE modelling has the advantage of allow a flexible regression framework (with easy comparison between tests), allowing for clustering of observations by participants and incomplete data without only requiring extensive distributional assumptions. The GEE modelling will be applied using the local FFA finding as the reference standard. It will be assumed that at time

points when index tests are available but the reference standard was not available, the result is negative until negative positive reference standard finding.

Analysis C. Person level diagnostic accuracy analyses using a combination of tests. Analysis C will use a combination of tests under a simple approach (i.e. positive if either index test is positive), instead of a single test under the diagnosis analysis approach. Specifically, the impact of combining OCT with fundus evaluation, Amsler test, patient's subjective assessment of vision, or visual acuity tests will be considered. For the "both positive" approach there will need to be a prior visit where both of the index tests were positive to define a positive result and there will be a negative result if there are no prior visits where both the index tests are positive. For the "either positive" approach there will need to be at least one prior positive index test result from either of the tests to define a positive result and no prior positive index test results from both of the tests to define a negative result. The reference standard and index tests result will match the definition of Analysis A1, using all valid index test results, with the additional step of generating an overall combination test result.

Analysis Objective 2: To develop a risk prediction model

Analysis D. Prognostic modelling. A risk prediction model using Cox regression will be developed to predict development of nAMD (using the clinical reference standard – see reference standard definition C above in table 3) in the EDNA study eye using baseline risk factors. Appendix 1 shows the table of risk factors which will be considered for inclusion in the model. The model's predictive performance will be assessed in terms of discriminative and calibrative ability. Discriminative ability will be assessed using Harrell's *c*-index. Calibration will be assessed by plotting the average predicted risk compared with the corresponding Kaplan-Meier estimate of the observed risk by tenth of predicted risk. Recalibration of the model will be undertaken via adding/removing predictors if necessary. Internal validation will be undertaken using a bootstrapping approach. (Moons 2012).

Analysis Objective 3: Quantify the time to and risk of conversion

Analysis E. time-to-conversion distribution. The survival distribution of conversion to nAMD (defined as local FFA conversion in a first analysis; and as the clinical diagnosis in a second analysis) over the follow-up period will also be estimated. Participants that did not convert will be censored at the time of their last available observation. First, a Kaplan-Meier curve will be fitted to estimate the underlying nAMD conversion distribution. Second, time of conversion will be estimated based upon the date of conversion confirmation. The distribution will be estimated

assuming parametric distributions and addressing the interval nature of the data. This will be done using the *stintreg* command in Stata. Exponential, Weibull and log-normal proportional hazard survival models will be fitted. Furthermore, using an accelerated time failure approach, a generalised gamma distribution will also be used. Related assessments of fit will be summarised.

Analysis Objective 4: To explore the impact of CNV subtype on time to conversion

Analysis F. Cox proportional hazard modelling.

For participants with the CNV subtype only an exploratory analysis of the hazard ratio for the covariate "percentage of classic" to explore association between the occult/classic mix and conversion.

Missing data and indeterminate and unreliable results

Absence of a positive reference standard during valid follow-up period with be presumed to indicate a negative result.

Indeterminate FFA results: As a default position indeterminate tests will be treated as missing, but there will be sensitivity analyses to test that assumption (see below). For the primary person level analysis (Analysis A1), if the last available reference standard is missing/indeterminate then the previous reference standard will be used. If no reference standards have been carried out after the baseline reference standard or they are all indeterminate/missing then that participant will be excluded from the analysis unless a curtailed period with a valid FFA and index test result exists.

For the prognostic model (Analysis D) the impact of missing covariate data will be assessed and multiple imputation will be used for baseline variables if considered appropriate.

Sensitivity analyses

For all the sensitivity analyses the reference standard is the local FFA and the index tests use the collapsed definition using all valid index test results as per analysis A1 in table 3.

A sensitivity analysis will use OCT from the reading centre (rather than local OCT) as the index test and calculate the diagnostic performance of OCT and the paired comparisons between the reading centre OCT and the other index tests (sensitivity analysis 1).

Sensitivity analyses will explore the impact of varying the test cut-off for relevant tests (varying positive test definition of the patient's subjective assessment to "worse" – sensitivity analyses 2, and of the visual acuity test to 20 letters –sensitivity analysis 3 and of a visual acuity drop of 10 letters or more from last FFA confirmed false positive – sensitivity analysis 4) to explore possible threshold effects.

Sensitivity analysis 5 will define indeterminate FFA as positive and sensitivity analysis 6 as negative, instead of the default position of missing. Sensitivity analyses 5 and 6 will only be undertaken if there are a substantial number of indeterminate results (minimum of 10%).

The sensitivity analyses described apply only to the primary person level analysis (analyses A) and will not be carried out for the GEE model.

For the survival analysis the impact of changing the assumption of the date of participants developing nAMD will be tested. In the default analysis this will be the date of the FFA confirming nAMD. In the sensitivity analysis this date will be altered to a time halfway between the positive FFA and the previous negative FFA.

The sensitivity analyses are listed in the table below:

Reference standard definition: A = Local FFA. Collapsed definition. Reference standard is positive if local FFA is converted at any time point and negative if it is not converted throughout.

Index test definition: A = Collapsed definition. Positive if there is any prior positive index test and negative if the index test is negative throughout. All index tests are used.

Table 4 Description of sensitivity analyses

S.A. number	Analysis	Reference standard definition of disease	Index test definition
1	Uses OCT from the reading centre. Diagnostic performance of OCT and a paired comparison of the OCT and the other tests	A	A: reading centre OCT
2	A positive subjective vision is defined as "worse" or "much worse". Diagnostic performance of subjective vision and a paired comparison of subjective vision and the other tests	A	A: Subjective vision "worse" or "much worse"
3	A positive visual acuity is a change of 20 letters or more. Diagnostic performance of visual acuity and a paired comparison of visual acuity and the other tests.	A	A: Visual acuity change of 20 letters or more
4	A positive visual acuity is a change of 10 letters from the last FFA confirmed false positive. Diagnostic performance of visual acuity and a paired comparison of visual acuity and the other tests.	A	A: Visual acuity change of 10 letters or more from the last FFA confirmed false positive
5	Indeterminate FFA and indeterminate index tests taken as positive. Diagnostic performance of the individual tests and a paired comparison of sensitivity and specificity between the tests.	A: indeterminate results defined as positive.	A: indeterminate results defined as positive.
6	Indeterminate FFA and indeterminate index taken as negative. Diagnostic performance of the individual tests and a paired comparison of sensitivity and specificity between the tests.	A: indeterminate results defined as negative.	A: indeterminate results defined as negative.

Subgroup analyses

We will undertake pre-planned subgroups evaluation according to type of AMD in the study eye (choroidal neovascularization (CNV) and Retinal angiomatous proliferation (RAP)) and concerning analysis A1. These subgroup analyses will be classified as exploratory and evaluated at the 2-sided 5% significance level. They will only be carried out on index test results status collapsed over the follow-up period with the local FFA as the reference standard following analysis 1. No formal paired comparisons will be carried out and the individual group sensitivity (with 95% CIs) will be calculated.

For participants with a CNV subgroup (in the -study eye) the proportion of classic will be quantified.

Other analyses

Summary of key characteristics at the time of conversion. For those participants with a positive local FFA, the area of neovascularization and the absolute visual acuity, and the drop in visual acuity from baseline will be simply summarised. Area of neovascularisation (total lesion area) is an indication of the severity of the disease. Similarly, these characteristics will be quantified for those with a positive reading centre FFA.

Safety data

Within the EDNA study we will record only any AE/SAE relating to collection of blood or FFA requested during involvement in the study. AEs relating to FFAs conducted prior to recruitment to the study will not be reported.

Any AE/SAE resulting from treatment to the nAMD eye during the study will not be recorded as an AE/SAE. Once an EDNA participant has nAMD in the study eye, or the end of follow-up, any subsequent AE or SAE will not be recorded.

In this study the only AE and SAE that are expected relate to the collection of blood and the FFA.

FFA related expected adverse events: These may be local skin irritation, development of erythematous lesions on the skin immediately after FFA and more generalised reaction to the FFA with pulmonary and or other systemic manifestations (anaphylaxis)

Blood collection expected adverse events: bruising and discomfort at the site of any puncture.

Most participants in the study will be elderly, and we anticipate that 50% will be >75 years at the time of recruitment. Therefore it is expected that a proportion of the cohort will die from causes unrelated to the study over the period of follow up. Deaths unrelated to the study procedures will not be recorded as SAEs but will be recorded within the CRF.

Appendix 1

Table of planned factors for prognostic analysis

Unit of analysis (EYE if applicable or PERSON)	**Name of factor**
PERSON	Age
PERSON	Raised blood pressure
PERSON	Smoking history
PERSON	Cardiovascular disease
PERSON	Diabetes
PERSON	Gender
PERSON	Nutritional supplements
PERSON	Family history of AMD
PERSON	BMI
FELLOW EYE	Type of wet AMD in fellow eye (CNV/RAP)
FELLOW EYE	Severity of nAMD from the baseline non-EDNA eye
EDNA eye	Previous cataract surgery
EDNA eye	VA at baseline in EDNA eye
EDNA eye	Type of drusen at baseline EDNA eye
EDNA eye	Maximum size of drusen at baseline EDNA eye
EDNA eye	Most frequent size of drusen at baseline EDNA eye
EDNA eye	presence of pigmentary abnormalities in the fundus of the study eye
EDNA eye	Retinal thinning present
EDNA eye	Choroid thinning
EDNA eye	ELM disruption
EDNA eye	EZ disruption

Appendix 2

Dummy tables

A set of dummy tables and figures are provided below. The formatting of the final tables and figures used may vary, and these should be considered illustrated of the general approach planned to present the data and analyses.

Table - Baseline characteristics

All participants (N=XX)						
	N	**Mean**	**SD**			
Age						
BMI						
	N	**n**	**%**			
Sex: Male						
History: hypertension						
History: cardiovascular disease						
Family history AMD						
Diabetes						
Nutritional supplements						
Smoking history: current						
Smoking history: ex-smoker						
Smoking history: never						
Ethnicity:						
Cataract present?						
Yes						
No						
Pseudophakic						
AREDS grade **(out of those with a Phakic study eye N=)**	**0**	**1**	**2**	**3**	**Not known**	
	n(%)	**n(%)**	**n(%)**	**n(%)**	**n(%)**	
• NS						
• Cortical						
• PSC						
Fundus examination:	**n**	**%**				
No nAMD						
Early nAMD						
Geographic atrophy						
Exudative nAMD						
OCT examination:	**n**	**%**				
No nAMD						
Early nAMD						
Late nAMD						
Amsler scotoma:	**n**	**%**				
Yes						
No						
	Study eye			**Non-Study eye**		
Visual acuity: Number of letters	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**

Drug used in non-study eye				**N**	**n**	**%**
Lucentis						
Eylea						
Avastin						

An identical baseline table for participants developing nAMD and another identical baseline table for participants who don't develop nAMD.

Table - Tests/reference standard performed (replicated for each of the tests if applicable)

Study period (years)	**Average number of visits (min)(max)**	**Total number of visits done**	**Fundus photography n(%)**	**Patient reported vision n(%)**	**Visual acuity n(%)**	**Amsler test n(%)**	**OCT n(%)**	**FFA n(%)**
Year 0-1								
Year 1-2								
Year 2-3								
Years 0-3								

Table - Local FFA reference standard at study exit/last known state

	n	**%**
Positive test		
Negative test		
Indeterminate test		
No reference standard available		
Missing		
• Died		
• Withdrew		
• Clinical diagnosis		
• Unknown		

Table – Clinical diagnosis reference standard at study exit/last known state

	n	**%**
Positive test		
Negative test		
Indeterminate test		
No reference standard available		
Missing		

Table – Time in the study

	Median in days (P25-P75), count
Overall follow-up	
Time until conversion	
Time until last known state when no conversion happened	

Table - Index test results (analysis 1)

	Median in days (P25-P75), count
Time between OCT and last FFA	
• For positive OCT	
• For negative OCT	
Time between Fundus test and last FFA	
• For positive Fundus	
• For negative Fundus	
Time between visual acuity test and last FFA	
• For positive visual acuity	
• For negative visual acuity	
Time between subjective vision test and last FFA	
• For positive subjective vision	
• For negative subjective vision	
Time between Amsler test and last FFA	
• For positive Amsler	
• For negative Amsler	

Table - Index test results (analysis 1)

Index test		**n**	**%**
OCT	Positive test		
	Negative test		
	Indeterminate test		
	Missing		
Fundus	Positive test		
	Negative test		
	Indeterminate test		
	Missing		
Visual acuity	Positive test		
	Negative test		
	Indeterminate test		
	Missing		
Subjective vision	Positive test		
	Negative test		
	Indeterminate test		
	Missing		
Amsler	Positive test		
	Negative test		

	Indeterminate test		
	Missing		

Table - Conversion visit – number correctly identifying conversion (Reference Standard A and index test A).

Index test	n	%
OCT		
Fundus		
Visual acuity		
Subjective vision		
Amsler		

Table - Number of trigger events.

Index test	n
OCT	
Fundus	
Visual acuity	
Subjective vision	
Amsler	

Table - Adverse events

Description event	Expected/unexpected	Related FFA	Related phlebotomy

Table - Serious adverse events

Description event	Expected/unexpected	Related FFA	Related phlebotomy

Flow Diagram under analyses 1 to 7:

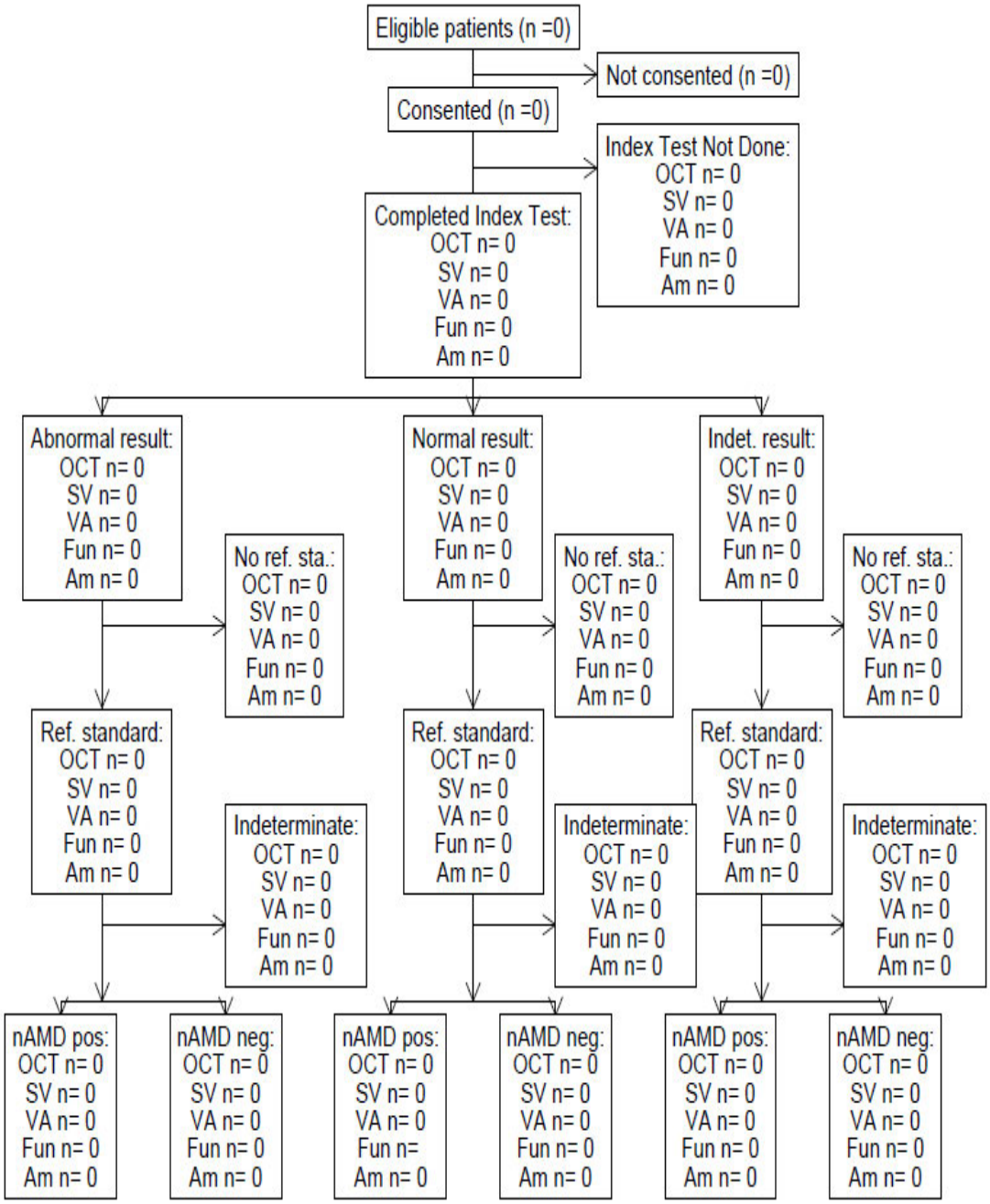

Table - Analysis 1:

Diagnostic performance of individual tests

Test	**Diagnostic parameter**	**Point estimate**	**Lower 95% CI**	**Upper 95% CI**
Visual acuity	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Prop. indeterminate tests			
OCT	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Prop. indeterminate tests			
Amsler test	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Prop. indeterminate tests			
Fundus evaluation	Sensitivity			
	Specificity			

	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Prop. indeterminate tests			
Subjective vision	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Prop. indeterminate tests			

Table - Analysis 1 continued:

Paired comparison of sensitivity and specificity between the tests

Tests compared	**Parameter**	**Test**	**Value(95% CI)**	**p-value (McNemar)**
Visual acuity vs OCT	sensitivity	visual acuity		
		OCT		
		difference		
	specificity	Visual acuity		
		OCT		
		difference		
Visual acuity vs Amsler	sensitivity	Visual acuity		
		Amsler		
		difference		
	specificity	Visual acuity		
		Amsler		

		difference		
Visual acuity vs fundus evaluation	sensitivity	Visual acuity		
		Fundus evaluation		
		difference		
	specificity	Visual acuity		
		Fundus evaluation		
		difference		
Visual acuity vs subjective vision	sensitivity	Visual acuity		
		subjective		
		difference		
	specificity	Visual acuity		
		subjective		
		difference		
OCT vs Amsler	sensitivity	OCT		
		Amsler		
		difference		
	specificity	OCT		
		Amsler		
		difference		
OCT vs fundus evaluation	sensitivity	OCT		
		Fundus evaluation		
		difference		
	specificity	OCT		
		Fundus evaluation		
		difference		
OCT vs subjective	sensitivity	OCT		
		subjective		

		difference		
	specificity	OCT		
		subjective		
		difference		
Amsler vs fundus evaluation	sensitivity	Amsler		
		Fundus evaluation		
		difference		
	specificity	Amsler		
		Fundus evaluation		
		difference		
Amsler vs subjective	sensitivity	Amsler		
		Subjective		
		difference		
	specificity	Amsler		
		Subjective		
		difference		
Fundus evaluation vs subjective	sensitivity	Fundus evaluation		
		Subjective		
		difference		
	specificity	Fundus evaluation		
		Subjective		
		difference		

Table - Sensitivity Analyses (same tables for all relevant sensitivity analyses):

Sensitivity Analysis – diagnostic performance of OCT from the reading centre.

Test	**Diagnostic parameter**	**Point estimate**	**Lower 95% CI**	**Upper 95% CI**
OCT	Sensitivity			
	Specificity			
	Positive likelihood ratio			

	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Indeterminate tests			

Table - Paired comparison of the sensitivity and specificity between the reading centre OCT and the other tests.

Tests compared	**Parameter**	**Test**	**Value(95% CI)**	**p-value (McNemar)**
OCT vs Visual acuity	sensitivity	OCT		
		Visual acuity		
		difference		
	specificity	OCT		
		Visual acuity		
		difference		
OCT vs Amsler	sensitivity	OCT		
		Amsler		
		difference		
	specificity	OCT		
		Amsler		
		difference		
OCT vs fundus evaluation	sensitivity	OCT		
		Fundus evaluation		
		difference		
	specificity	OCT		
		Fundus evaluation		
		difference		
OCT vs subjective	sensitivity	OCT		
		subjective		
		difference		

	specificity	OCT		
		subjective		
		difference		

Table 18 - Subgroup analysis – single test – analysis for the subgroups according to the type of AMD i.e. CNV, RAP and PCV

Subgroup	Test	Diagnostic parameter	Point estimate	Lower 95% CI	Upper 95% CI
CNV	Visual acuity	Sensitivity			
	OCT	Sensitivity			
	Amsler test	Sensitivity			
	Fundus evaluation	Sensitivity			
	Subjective vision	Sensitivity			
RAP	Visual acuity	Sensitivity			
	OCT	Sensitivity			
	Amsler test	Sensitivity			
	Fundus evaluation	Sensitivity			
	Subjective vision	Sensitivity			
PCV	Visual acuity	Sensitivity			
	OCT	Sensitivity			
	Amsler test	Sensitivity			
	Fundus evaluation	Sensitivity			
	Subjective vision	Sensitivity			

Table - Combination of diagnostic tests – treating an overall positive result as one or more positive results in individual tests

Tests	Diagnostic parameter	Point estimate	Lower 95% CI	Upper 95% CI
OCT & Visual acuity	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			

	Diagnostic odds ratio			
	Indeterminate tests			
OCT & Amsler	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Indeterminate tests			
OCT & fundus evaluation	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Indeterminate tests			
OCT & subjective vision	Sensitivity			
	Specificity			
	Positive likelihood ratio			
	Negative likelihood ratio			
	AUC			
	Diagnostic odds ratio			
	Indeterminate tests			

Table - Timing of conversion to nAMD-primary
(Conversion assumed to be at the time of the first positive FFA test)

Time	Beg. total	Fail	Net lost	Survival probability
1				
2				
3				
4				
5				

6				
7				
8				
9				
10				
etc.				

Summary statistics of the area of neovascularisation for FFA positive participants using the local FFA

Definition of conversion	**Measure**	**Mean (SD)**	**Median (IQR)**	**Range**
Local FFA	**Area**			
	Visual acuity absolute level			
	Visual acuity drop from baseline			
Reading Centre FFA	**Area**			
	Visual acuity absolute level			
	Visual acuity drop from baseline			

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