Effectiveness of Multimodal imaging for the Evaluation of Retinal oedema And new vesseLs in Diabetic retinopathy (EMERALD)

Statistical Analysis Plan

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CONTENTS

1. INTRODUCTION

This document details the proposed data presentation and analysis for the final study report from **EMERALD (Effectiveness of Multimodal imaging for the Evaluation of Retinal oedema And new vesseLs in Diabetic retinopathy). EMERALD is a HTA-funded, prospective, case-referent cross-sectional diagnostic study investigating diagnostic performance and cost-effectiveness of a new form of surveillance (ophthalmic grader pathway) for people with stable diabetic macular oedema (DMO) and/or proliferative diabetic retinopathy (PDR).**

The results reported in the main paper(s) and final study reports will follow the strategy set out within this document. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they will follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis, nor to prohibit accepted practices, but establish the rules that will be followed, as closely as possible, when analysing and reporting the study.

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

Any substantive deviations from the statistical analysis plan will be described and justified in the final report of the study. The analysis will be carried out by an identified, appropriately qualified and experienced statistician, who will ensure the integrity of the data during their processing.

1.1 Key personnel

Author: Jonathan Cook (Senior Statistician)

Approver: Jonathan Cook (Senior Statistician)

Approver: Noemi Lois (Chief Investigator)

1.2 Changes from previous version of Statistical Analysis Plan

The table below summarises of key changes from earlier versions of statistical analysis plan (SAP), with particular relevance to protocol changes that have an impact on the design, definition, sample size, data quality/collection and analysis of the outcomes will be provided.

Version number Issue date	Author of this issue	Protocol Version & Issue date	Significant changes from previous version together with reasons
V0.3_01Feb2019	WS	V3.0 19 March 2017	Not applicable as this is the 1st issue

2. BACKGROUND AND OBJECTIVES

2.1 Background and rationale

Diabetic Retinopathy (DR) is the most common microvascular complication of diabetes mellitus and a leading cause of visual loss among individuals of working age (Zheng *et al.* 2012, Liew *et al.* 2014). Patients with DR may lose sight as a result of the development of diabetic macular oedema (DMO) and/or proliferative diabetic retinopathy (PDR), the major complications of DR.

In DMO fluid accumulates in the central part of the retina, the macula, which is responsible for detailed central vision whilst in PDR, abnormal new blood vessels ("new vessels") grow on the optic nerve head or on the surface of the retina and towards the inside of the eye.

Furthermore, DMO and PDR may be considered active or inactive. Active DMO can be defined as a central subfield retinal thickness (CRT) of > 300 microns and/or presence of intraretinal/subretinal fluid on spectral domain OCT, whilst active PDR can be considered to be present in eyes with sub-hyaloid/vitreous haemorrhage and/or active new vessels.

Currently in the NHS ophthalmologists with expertise in retinal diseases assess patients during follow up visits.

Patients with DMO are currently evaluated during follow-up in clinic using a visual acuity test, most often undertaken by a nurse; optical coherence tomography (OCT), obtained by a photographer and interpreted by the ophthalmologist, and slit-lamp biomicroscopy, undertaken by an ophthalmologist. In the follow-up of patients with PDR ophthalmologists typically examine the patient by slit-lamp biomicroscopy.

Given the high number of people with DMO and PDR, the need for patients to be seen at short follow-up intervals to determine whether patients remain stable or whether their disease reactivates requiring treatment and the need for a life-long follow-up given the chronic nature of the disease, there is a very large workload in Hospital Eye Services related to DMO/PDR.

2.2 Objectives

The aim of this study is to determine the diagnostic performance and cost-effectiveness of a new form of surveillance of people with stable DMO and/or PDR, taking as reference standard the current standard of care (for DMO: ophthalmologist evaluating patients in clinic by slit-lamp biomicroscopy and with access to OCT images; for PDR ophthalmologists evaluating patients in clinic by slit-lamp biomicroscopy). The new form of surveillance will entail multimodal retinal imaging obtained by trained photographers/imaging technicians and separate image assessment by trained graders. This new surveillance pathway will need to be investigated separately for DMO and PDR as these conditions are diagnosed, treated and followed-up differently (though they are often present in the same individual and even in the same eye).

Diagnostic performance will be determined within the study. All patients will partake in the standard care pathway in which assessment will be performed through a face-to-face examination by an ophthalmologist (ophthalmologist evaluating the patient in clinic using slit-lamp biomicroscopy, and with access to OCT scans, as done in routine clinical practice). Images including OCTs, as done during routine clinical practice, and 7 field ETDRS images and ultra-wide field images (sometimes referred to as wide angle fundus) which will be done specifically for the purpose of EMERALD, will be evaluated by trained ophthalmic graders. In addition, the 7 field ETDRS images and the ultra-wide field fundus images will be also assessed by ophthalmologists. A comparison will be performed to determine the diagnostic performance of detecting active DMO/PDR assessed by the ophthalmic grader against the *reference standard* (ophthalmologist slit-lamp biomicroscopy assessment with access to OCTs). For patients with PDR, an *enhanced reference standard* (evaluation of the patient by the ophthalmologist using slit-lamp examination supplemented by ophthalmologist evaluation of 7

field ETDRS/ultra-wide fundus images) will also be used in a sensitivity analysis. 7 field ETDRS and ultra-wide field fundus images will be evaluated independently of one another for this purpose.

Specifically, objectives of this study are to evaluate the new surveillance pathway in terms of:

1. Quantify the diagnostic accuracy (in terms of sensitivity, specificity, overall agreement, positive and negative likelihood ratios) of the new pathway of surveillance (ophthalmic grader pathway) using the current standard of care pathway as the reference standard. This will be done separately for DMO and PDR. For PDR only, the diagnostic accuracy using the 7 fields versus Ultra Wide angle imaging will be compared. Furthermore, for PDR, an enhanced reference standard (see below for details) will also be used to quantify the diagnostic accuracy.
2. Acceptability of the new surveillance pathway.
3. Proportion of patients requiring subsequent full clinical assessment by an ophthalmologist under the new pathway.
4. Proportion of patients unable to undergo imaging tests, with images of inadequate quality and indeterminate findings under the new pathway.
5. Relative cost-effectiveness of the new surveillance pathway.

The SAP covers the analysis strategy for objectives 1, 3 and 4. Objectives 2 and 4 will be addressed by the qualitative analysis and health economic analyses respectively.

3. STUDY METHODS

3.1 Trial Design/framework

EMERALD is a prospective, cross-sectional diagnostic study of patients with diabetic retinopathy and DMO or PDR (or both) who had been previously successfully treated and who, at the time of enrolment in the study, may have active or inactive disease (both are required to evaluate the diagnostic performance of the new pathway).

Specifically, EMERALD will have a case-referent cross-sectional diagnostic study design with both sampling (selection) of patients and data collection carried out prospectively (Knottnerus & Muris, 2003). This approach provides both a cost-efficient study design while also having a low risk of bias in terms of diagnostic accuracy (Whiting *et al.* 2011).

When patients with previously successfully treated DMO/PDR attend clinic they will be assessed as per standard practice. They will undergo visual acuity testing, OCT and fundus examination, and undergo Ophthalmologist evaluation to (1) confirm eligibility (2) obtain informed consent, and (3) determine whether active/inactive DMO/PDR is present or not.

In addition, patients enrolled in EMERALD will undergo 7 field ETDRS and ultra-wide field fundus images.

Diagnostic performance of the EMERALD ophthalmic graders will be assessed against the Ophthalmologist assessment reference standard) to determine:

- **Sensitivity** (the proportion of patients determined by the Ophthalmologist to suffer from active DMO/PDR which have been correctly identified by EMERALD ophthalmic graders),
- **Specificity** (the proportion of patients determined by the Ophthalmologist to suffer from inactive DMO/PDR which have been correctly identified by EMERALD ophthalmic graders),
- **Overall agreement** (a measure of how well ophthalmic graders assessment agree with the Ophthalmologist assessment), along with

- **Positive likelihood ratio** (the probability a patient with active DMO/PDR being correctly assessed by the ophthalmic graders, divided by the probability a patient with inactive DMO/PDR being incorrectly assessed by the ophthalmic graders as being active and
- **Negative likelihood ratio** (the probability a patient with active DMO/PDR being incorrectly assessed by the ophthalmic graders as being active, divided by probability a patient with inactive DMO/PDR being correctly assessed).

For PDR only, an enhanced reference (also incorporating ultra-wide field image) will be used for some analyses.

3.2 Randomisation and Masking

Masking

The ophthalmic grader interpreting patients' images (OCT, 7 field ETDRS fundus and ultra-wide images) will be masked to the reference standard. To assure masking, ophthalmic graders will not be interpreting images from patients recruited at their own centre and will not have access to the reference standard. Furthermore, they will not know from which patients 7 field ETDRS fundus images, ultra-wide field fundus images or OCTs come from and will not read 7 field ETDRS fundus images, ultra-wide field fundus images or OCTS of the same patient, to ensure that their reading of one imaging technology will not influence their reading of the other.

Ophthalmologists doing the standard of care evaluation will also be masked to the findings/decisions made by the ophthalmic graders (who will be reviewing the images at a later date). Ophthalmologists reading the fundus photographs (7 field ETDRS and ultra-wide field fundus images) for the purpose of evaluating the alternative "enhanced" reference standard will also be masked in the same manner as the ophthalmic graders (they will not assess images obtained in their own centres, to ensure they are masked to the result of the reference standard and not influenced by it and will not be aware of the ophthalmic graders assessment, which will not be made accessible to them; they will not be grading 7 field ETDRS and ultra-wide angle fundus images of the same patient either).

Patients will also be masked to findings/decisions made by the ophthalmic graders (these will not be available at the time of the study's clinical visit). Patients will not be masked to the decisions made in the standard of care pathway as these will guide their care. However, this should not introduce any bias as the photographer/imaging technicians obtaining the images will be different from the ophthalmic graders interpreting the images (i.e. the ophthalmic graders evaluating the images for the proposed new care pathway will be from a different institution than that where the patients will be evaluated).

Randomisation

Due to the diagnostic accuracy focus of the EMERALD study, patients will not be randomised for assessment by Ophthalmologists.

Ophthalmic images will be anonymised and uploaded electronically to the Central Angiographic Resource Facility (CARF) via their Secure File Transfer Protocol (SFTP) service. On receipt, CARF will log the images received and assign a randomised number to each participant so that ophthalmic graders and ophthalmologists will not be aware at which EMERALD site the images were captured. Each full set of participant images will then be divided by imaging modality and by eye in order that they may be assessed by six different ophthalmic graders and four different ophthalmologists (each receiving only one modality/eye pairing per participant).

At each review stage, all images available for grading will be divided equally between the number of ophthalmic graders and ophthalmologists assigned to the study. As each ophthalmic grader or ophthalmologist cannot assess images obtained at their own centres and cannot assess more than one modality/eye pairing per participant, CARF will log each modality/eye pairing as it is assigned. At each subsequent review, the log will be checked to ensure that images belonging to a participant are not assigned to the same reviewer for assessment.

CARF will then upload all assigned images to a designated EMERALD imaging server. This server, established by CARF and sitting within their Data Centre, hosts the OphthalSuite imaging review platform which will be used to assess the study images. Each ophthalmic grader and ophthalmologist will be assigned a username and password for remote authentication to the server and to OphthalSuite, which will authorise them to access only the images specifically assigned to them for review.

Graders will assess images for individual eyes (not as a pair for the same person), and will assess whether active/inactive disease is present, the location (for DMO whether or not it is involving the centre of the macula; for PDR the location of new vessels in the fundus), and whether they require referral to an ophthalmologist.

For the secondary analyses addressed at the eye level, a random eye will be selected using a random uniform number (0 to 1) generated in Stata where <0.5 the left eye is used and 0.5 or above the right eye is used. If reference standard data is only available for one eye, that eye will be used irrespective of the random number.

3.3 Sample Size

The sample size was determined upon the basis of setting a target of the number of reactivated (active) DMO and PDR patients which would enable sensitivity to be tested against a pre-specified target level of 80%. The required sample size was calculated using formula T1 from Obuschowski(Obuchowski, 1998) in Microsoft Excel – it was a Wald-test based calculation. This level was considered the minimum acceptable level for the new pathway (ophthalmic grader pathway) to be clinically viable. A lower specificity is considered acceptable and a target of 65% for specificity was used to confirm sufficiency of the sample size for assessing specificity. However, it should be noted that the actual specificity level which would be acceptable in practice is uncertain as in reality this would be driven by cost-effectiveness and resource availability considerations which may make a substantially lower specificity still viable, because it would still result in saving of ophthalmologist time. In such a scenario this calculation may be conservative. To be able to detect if the sensitivity of the new pathway (photographer/imaging technician pathway) is 10% and 12% higher than the 80% minimal target set) with 80% and 90% power would require 89 participants with DMO/PDR who have reactivated (active DMO/PDR), with 2-sided 5% significance level (Silva *et al.* 2015). 93 participants who have not reactivated (inactive DMO/PDR) would enable a specificity 15% higher than the 65% target to be detected with 90% power. A 95% confidence interval for photographer sensitivity and specificity would have a confidence interval (Wilson method) with a width of 10-20% depending on the observed level (Piegorsch, 2004). Allowing for 10% missing/indeterminate results, 104 individuals who have re-activated and 104 who have not, are required (208 for each, DMO and PDR) which leads to a need for a maximum of 416 participants in the study overall; some participants may have both existing DMO and PDR thus contributing to both the DMO and the PDR targets.

3.4 Statistical Interim Analysis, Data Review and Stopping guidelines

All participants taking part in this study for generating the **reference standard** will receive standard NHS care. No participants will be placed in a greater state of risk when taking part in this study. As a result a Data and Safety Monitoring Committee was not considered necessary.

No formal interim analyses or stopping guidelines have been planned.

3.5 Timing of Final Analysis

EMERALD aims to recruit a maximum of 416 patients with previously successfully treated and stabilised DMO and/or PDR in one or both eyes at the time of enrolment into the study to establish the **reference standard**.

- 104 in whom DMO is active
- 104 in whom DMO is inactive

Total = minimum of 208 patients with DMO

- 104 in whom PDR is active
- 104 in whom PDR is inactive

Total = minimum of 208 patients with PDR

3.6 Statistical Analysis Outline

Outcomes for the DMO and PDR patients will be assessed in separate analyses under the principal analyses. This is in keeping with the approach adopted for the sample size and how recruitment to the study was conducted where DMO and PDR had separate targets.

Participants will be categorised as having active DMO/inactive DMO/no DMO (active DMO = DMO currently present; inactive DMO = DMO was present in the past, was treated and remains now absent [inactive]; no DMO = patient had never had DMO in the past and there is no DMO present now). Similarly, they will also be categorise as active PDR/inactive PDR/no PDR (active PDR = active PDR currently present; inactive PDR = PDR was present in the past, was treated and remains now inactive; no PDR = patient had never had PDR in the past and there is no PDR present now) according to the diagnosis established at the standard care pathway at the person level (i.e. using data from both eyes). Active & inactive DMO/PDR will be subcategorised as previously successfully treated or not (for eligibility purposes, all patients would need to have at least one eye with one of the diseases – DMO or PDR – previously successfully treated for entering into the study). For active DMO/PDR there will be a further subdivision of non-previously successfully treated into existing DMO/PDR not successfully treated and de novo DMO/PDR. Each eye for all EMERALD participants will be assessed with regards to the status of PDR and DMO irrespective of whether they are "eligible" for both cohorts (see below for clarification of the meaning). Which data is included varies according to the analysis (see Tables 1 and 2 for a full list). "All patients" includes all of the possible diagnoses described above where as "eligible" for the new pathway is restricted to the relevant subset.

Those which had previously successfully treated DMO/PDR constitute "eligible" patients for the new pathway. Note, this use of the term "eligible" and elsewhere in this document does not refer to eligibility to the study. This person based assessment reflects the consequences of the clinical decision under the main analyses. Data is being collected for both eyes irrespective of the condition per DMO and PDR. As such an individual may not be eligible for the new pathway in terms of DMO or PDR in that they are eligible for the study due to, for example, their DMO status in the right eye, but they may not have any PDR (let alone successfully controlled PDR) in either eye (so are "ineligible" for some of the PDR analyses). Eligibility for the new pathway at an eye level will be category of previously successfully treated DMO/PDR. At the person level having one eye eligibility was lead to the person being categorised as eligible irrespective of the status of the other eye.

A graders decision upon whether to refer the patient or not, an inherently person level decision, will be used, rather than an assessment of the single eye. Furthermore, to more closely reflect how the new pathway would function in practice, a patient whom graders have classified DMO or PDR within the eye under the category "unsure" will be considered alongside those classified as "active" as both would be anticipated to require further examination by an ophthalmologist.

Sensitivity analyses will include assessment of the impact of the "unsure" test classification, more severe disease (DMO involving the centre of the macula or not; PDR with pre-retinal and/or vitreous haemorrhages indicating high risk characteristics) and of the ophthalmic grader's grade upon the diagnostic performance (see Section 4.3 below). The impact of using ultra-wide field imaging (OPTOS) instead of standard imaging (7 field ETDRS images) on the diagnostic performance of the new pathway will also be assessed under the principal analyses for PDR detection. In addition, for PDR, a sensitivity analysis will assess the diagnostic performance of the ophthalmic grader against the alternative "enhanced" reference standard (ophthalmologist slit-lamp biomicroscopy assessment supplemented by ophthalmologist evaluation of 7 field ETDRS / ultra-wide field fundus images) to detect active PDR.

Sensitivity, specificity, positive and negative likelihood ratios will be calculated (with appropriate 95% confidence intervals (CIs) for the alternative strategy using the current standard of care pathway findings as the reference standard. Agreement (concordance) between the new pathway and current standard of care pathway will also be calculated (with 95% Wilson CI) (Piegorsch, 2004). The difference in sensitivity and specificity between wide-angle and 7 field ETDRS fundus images assessed by the ophthalmic graders will be compared with corresponding 95% CIs produced using Newcombe's method for paired data (Newcombe, 1998).

The proportion of patients requiring subsequent full clinical assessment or unable to undergo assessments, with inadequate quality images or indeterminate findings will be calculated for the alternative pathway with corresponding CIs.

4. ANALYSIS

The principal analysis will be carried out with DMO and PDR patients assessed in two separate sets of analyses at the person level one for each disease (See Table 1). Analyses will be focussed upon the patient considered eligible for the new pathway by virtue of having had controlled disease (previously successfully treated) in one or both eyes ("Patients eligible for new pathway"). The main analysis will be according to the ophthalmic graders decision to refer to an ophthalmologist (irrespective of the reason). The *reference standard* will be the ophthalmologist assessment of the presence of the active form of the respective disease in either eye. This reflects the consequences of the clinical decision. The diagnostic performance of the new pathway will be quantified and compared with the standard care pathway. For PDR there will be two sets of results one using Ultra-wide field OPTOS base assessment and one using the 7 fields based assessment. The impact of using ultra-wide field imaging (OPTOS) instead of standard imaging (7 field ETDRS images) on the diagnostic performance of the new pathway will also be formally compared under the main analysis and also for SENA5 (see below) only.

4.1 Outcome Definitions

Table 1 List of principal analyses (main and sensitivity) of diagnostic accuracy

Analysis name	Level of Analysis	DMO index test positive	DMO reference standard	PDR index test positive	PDR reference standard	Population
Main	Person	OCT based ophthalmic grader referral for either eye	Ophthalmologists assessment of active DMO in either eye	Ultra-wide field OPTOS based ophthalmic grader referral/ 7 fields ETDRS based ophthalmic grader referral for either eye	Ophthalmologists face-to-face assessment of active PDR in either eye	Eligible patients for new pathway*
SENA1	Person	OCT based ophthalmic grader identification of active disease in either eye	Ophthalmologists assessment of active DMO in either eye	Ultra-wide field OPTOS based ophthalmic grader identification of active disease in either eye/ 7 fields ETDRS based ophthalmic grader identification of active disease in either eye	Ophthalmologists face-to-face assessment of active PDR in either eye	Patients eligible for new pathway*
SENA2	Person	OCT based ophthalmic grader referral for either eye	Ophthalmologists assessment of active DMO in either eye requiring treatment	Ultra-wide field OPTOS based ophthalmic grader referral for either eye/7 fields ETDRS based ophthalmic grader referral for either eye	Ophthalmologists face-to-face assessment of active PDR in either eye requiring treatment	Patients eligible for new pathway*
SENA3	Person	OCT based ophthalmic grader	Ophthalmologists assessment of central involving	NA	NA	Patients eligible for

		identification of central involving DMO in either eye	DMO in either eye			new pathway*
SENA4	Person	NA	NA	Ultra-wide field OPTOS based ophthalmic grader referral for either eye/ 7 fields ETDRS based ophthalmic grader referral for either eye	Ophthalmologists face-to-face assessment of active PDR with pre-retinal or vitreal haemorrhaging in either eye	Patients eligible for new pathway*
SENA5	Person	NA	NA	Ultra-wide field OPTOS based ophthalmic grader referral for either eye/ 7 fields ETDRS based ophthalmic grader referral for either eye	Ophthalmologists face-to-face assessment combined with separate ophthalmologist assessment of 7 fields ETDRS and OPTOS wide-angle images of active PDR in either eye	Patients eligible for new pathway*
SENA6	Person	OCT based ophthalmic grader referral for either eye [participants assessed in routine clinic setting only]	Ophthalmologists assessment of active DMO in either eye [participants assessed in routine clinic setting only]	Ultra-wide field OPTOS based ophthalmic grader referral for either eye [participants assessed in clinical sites only]/7 fields ETDRS based ophthalmic grader referral for	Ophthalmologists face-to-face assessment of active PDR in either eye [participants assessed in routine clinic setting only]	Patients eligible for new pathway*

				either eye [participants assessed in routine clinic setting only]		

* Eligible patients for new pathway refer to the presence of previously successfully controlled active disease (DMO or PDR respectively) in one or both eyes. Those with uncontrolled disease, de novo disease, or no disease in one eye will not be included in the analysis of the respective disease (DMO or PDR) aimed at this population unless the other eye has previously successfully controlled disease for that disease.

4.2 Analysis Methods

For all diagnostic accuracy analyses, the sensitivity, specificity, positive and negative likelihood ratios will be calculated (with appropriate 95% confidence intervals (CIs)). The difference in sensitivity and specificity between ultra-wide field and 7 field ETDRS fundus images assessed by the ophthalmic graders will be compared with corresponding 95% CIs produced using Newcombe's method for paired data (Newcombe, 1998) and McNemar's test (McNemar, 1947).

4.3 Sensitivity analyses of the principal analysis of the diagnostic accuracy

In addition to the primary analysis for DMO and PDR, a number of sensitivity analyses will be conducted (listed in Table 1).

First, SENA1 will assess of the impact of the "unsure" test classification and of the ophthalmic grader's grade upon the diagnostic performance by defining the index test positive as definite assessment of the active disease (as opposed to allowing referral for "unsure" or quality of images). SENA2 will assess the ophthalmic graders referral assessments against the ophthalmologist assessment of those requiring treatment for both DMO and PDR. The third sensitivity analysis (SENA3) will focus on diagnostic performance for assessment amongst patients considered to possess the most severe form of DMO, central retina involving macular oedema. For PDR only, a sensitivity analysis (SENA4) will assess the diagnostic performance of the ophthalmic grader against the ophthalmologist slit-lamp biomicroscopy assessment to detect active PDR with pre-retinal or vitreous haemorrhage. For PDR only, a sensitivity analysis (SENA5) will assess the diagnostic performance of the ophthalmic grader against the alternative *enhanced reference standard* (ophthalmologist slit-lamp biomicroscopy assessment supplemented by ophthalmologist evaluation of 7 field ETDRS / ultra-wide field fundus images) to detect active PDR. A further sensitivity analysis (SENA6) will assess diagnostic accuracy for only a subset of participants who were assessed in a "typical" NHS clinic setting as opposed to a research clinic but otherwise under the same conditions as the main analysis.

4.4 Secondary analyses of diagnostic accuracy

In addition to the principal analyses which will be conducted at the person level separately for DMO and PDR, two distinct secondary analyses of diagnostic accuracy are planned (See Table 2 for a full list). Both will focus upon the entire EMERALD patient population ("All patients"). First, a limited set of eye level analyses will be carried out using the positive identification of active disease at the eye level (SECA1A-C). A random eye will be selected where two eyes are eligible with ophthalmic grader eye specific assessment against the corresponding ophthalmologist assessment. Second, on a similar scale, person level analyses will be carried out of the overall referral status of a patient irrespective of whether it is active DMO or PDR that requires

referral (SECA2A-C). SECA2A will utilise an ophthalmic grader referral assessment for either disease; for DMO OCT assessment will be use and for PDR the Ultra-wide field OPTOS or the 7 fields assessment in turn (i.e. two set of results). SECA2B is the same as SECA2A except that a visual acuity poorer than 6/12 would also be considered a valid reason for referral. SECA2C is the same as SECA2A except the reference standard is an ophthalmologist's assessment of the presence or not of active disease which requires treatment. The combined ophthalmic assessment of DMO or PDR in either eye will be reference standard based upon the Ophthalmic assessment. Diagnostic performance for both secondary analyses will be assessed using the same outcomes and methods as for the principal analyses.

Table 2 List of secondary analyses of diagnostic accuracy

Analysis name	Level of Analysis	DMO index test positive	DMO reference standard	PDR index test(s) positive	PDR reference standard	Population
SECA1A	Eye	OCT based ophthalmic grader assessment of active DMO in the randomly selected eye	Ophthalmologists assessment of active DMO in the randomly selected eye	Ultra-wide field OPTOS based ophthalmic grader assessment of active PDR/ 7 fields ETDRS based ophthalmic grader assessment of active PDR	Ophthalmologists face-to-face assessment of active PDR in the randomly selected eye	All patients†
SECA1B	Eye	OCT based ophthalmic grader identification of central involving DMO in either eye	Ophthalmologists assessment of central involving DMO in either eye			All patients†
SECA1C	Eye	NA	NA	Ultra-wide field OPTOS based	Ophthalmologists face-to-face assessment	All patients†

				ophthalmic grader referral/ 7 fields ETDRS based ophthalmic grader referral	combined with ophthalmologists assessment of 7 fields ETDRS and OPTOS wide-angle images of active PDR in either eye	
Analysis name	**Level of Analysis**	**Combined DMO/PDR test positive**		**Combined DMO/PDR reference standard**		**Population**
SECA2A	Person	Ophthalmic grader referral based upon OCT for DMO and either Ultra-wide field OPTOS based ophthalmic grader referral or 7 fields ETDRS based ophthalmic grader referral for PDR [referral for either disease will be considered a referral for the combined test]		Ophthalmologists face-to-face assessment of active DMO or active PDR in either eye		All patients†
SECA2B	Person	Ophthalmic grader referral based upon OCT for DMO and Ultra-wide field OPTOS based ophthalmic grader referral/7 fields ETDRS based ophthalmic grader referral for PDR, & Visual acuity >6/12 (or ETDRS equivalent letter) [referral for either disease, or due to visual		Ophthalmologists face-to-face assessment of active DMO or active PDR in either eye		All patients†

		acuity will be considered a referral for the combined test]		
SECA2C	Person	Ophthalmic grader referral based upon OCT for DMO and either Ultra-wide field OPTOS based ophthalmic grader referral or 7 fields ETDRS based ophthalmic grader referral for PDR [referral for either disease, or due to visual acuity will be considered a referral for the combined test]	Ophthalmologists face-to-face assessment of active DMO or active PDR in either eye requiring treatment	All patients†

† The "All patients" population will include in the analysis those with uncontrolled disease, de novo disease, or no disease in one eye irrespective of the disease status of the other eye, as well as those who are eligible patients for new pathway.

4.5 Supplementary/ Additional Analyses and Outcomes

The proportion of patients requiring subsequent full clinical assessment or unable to undergo assessments due to inadequate quality images or indeterminate findings will be calculated for the alternative pathway with corresponding CIs (with 95% Wilson CI). Agreement (concordance) between the new pathway and current standard of care pathway will also be calculated (with 95% Wilson CI; Piegorsch, 2004).

5. SPECIFICATION OF STATISTICAL PACKAGES

All analysis will be carried out using appropriate validated statistical software such as STATA (StataCorp., 2017) or R (R Core Team, 2018). The relevant package and version number will be recorded in the Statistical report.

6. REFERENCES

Knottnerus JA, Muris JW. Assessment of the accuracy of diagnostic tests: the cross-sectional study. Journal of Clinical Epidemiology 2003;56(11):1118–28.

Liew G, Michaelides M, Bunce C. A comparison of the causes of blindness certifications in England and Wales in working age adults (16-64 years), 1999-2000 with 2009-2010. BMJ Open. 2014 Feb 12;4(2):e004015.

McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika 1947;12:153–7. http://dx.doi.org/10.1007/BF02295996

Newcombe RG. Improved confidence intervals for the difference between binomial proportions based on paired data. Stat Med 1998;17:2635–50. http://dx.doi.org/10.1002/(SICI)1097-0258(19981130)17:22<2635::AID-SIM954>3.0.CO;2-C

Obuchowski NA. Sample size calculations in studies of test accuracy. Statistical Methods in Medical Research 1998; 7: 371-392.

Piegorsch WW. Sample sizes for improved binomial confidence intervals. Computational Statistics & Data Analysis 2004;46:309-16.

Silva PS et al. Real-Time Ultrawide Field Image Evaluation of Retinopathy in a Diabetes Telemedicine Program. Diabetes Care. 2015 Sep;38(9):1643-9.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.R-project.org

StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC

Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JAC, and Bossuyt PMM. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. Ann Intern Med 2011;155(8):529-36.

Zheng Y, He M, Congdon N. The worldwide epidemic of diabetic retinopathy. Indian J Ophthalmol. (2012) 60:428-31. doi:10.4103/0301-4738.100542.

APPENDIX: GLOSSARY OF ABBREVIATIONS

CARF	Central Angiographic Resource Facility
CI	Confidence Interval
CRT	Central Subfield retinal Thickness
DMO	Diabetic Macular Oedema
DR	Diabetic Retinopathy
EMERALD	Effectiveness of Multimodal imaging for the Evaluation of Retinal oedema And new vesseLs in Diabetic retinopathy
OCT	Optical Coherence Tomography
PDR	Proliferative Diabetic Retinopathy
SAP	Statistical Analysis Plan
SECA	SECondary Analysis
SENA	SENsitivity Analysis
SFTP	Secure File Transfer Protocol