STATISTICAL ANALYSIS PLAN

Study Title: **Cha**racterising disease **M**echanisms in **P**atients with **C**oronary **M**icrovascular **D**isease (**ChaMP-CMD**)

Study Funder: Medical Research Council

Study Registration Number: ISRCTN94728379

Trial statistician: Dr. Abdel Douiri

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Glossary

ANOCA	Angina with unobstructed coronary arteries
CBF	Coronary blood flow
CCS	Canadian Cardiovascular Society
CFR	Coronary flow reserve
CMD	Coronary microvascular disease
CMR	Cardiac magnetic resonance
ETT	Exercise treadmill test
fCMD	Functional coronary microvascular disease
hMR	Hyperaemic microvascular resistance
LVEF	Left ventricular ejection fraction
METs	Metabolic equivalent
MPRI	Myocardial perfusion reserve index
NYHA	New York Heart Association
QOL	Quality of life
SAQ	Seattle Angina Questionnaire
sCMD	Structural coronary microvascular disease

Table of Contents

1 Introduction

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting of the clinical trial entitled 'Characterising disease mechanisms in patients with coronary microvascular disease' (ChaMP-CMD). This is a single centre prospective randomised controlled trial designed to assess the utility of coronary physiology assessment in predicting response to anti-ischaemic therapy in patients with angina and non-obstructive coronary arteries (ANOCA).

The following documents were reviewed in preparation of this SAP:

• ChaMP-CMD trial protocol version 6.0, dated 29.09.2022

2 Study background

Stable exertional angina can be due to a flow limiting atheromatous plaque in the epicardial coronary arteries or due to dysfunction of the coronary microvasculature. Up to 50% of patients with angina have ANOCA, which comprises several distinct pathophysiological entities, such as coronary microvascular disease (CMD) and coronary artery spasm. CMD is diagnosed when there is an inadequate augmentation in coronary blood flow (CBF) in response to adenosine which is quantitatively expressed as coronary flow reserve (CFR); a diagnostic CFR threshold of 2.5 has the optimal sensitivity and specificity to detect a substrate for myocardial ischaemia and coronary perfusion inefficiency in response to exercise. It has recently been demonstrated that CMD itself may be a heterogeneous condition comprising two distinct subtypes (functional and structural CMD). Functional CMD is characterised by an elevated resting baseline CBF, due to vasodilation at rest, leading to a suboptimal vasodilatory capacity in response to stress, despite normal microvascular resistance. By contrast, structural CMD is characterised by elevated minimal microvascular resistance, which in these patients is the main determinant of diminished vasodilator capacity in response to stress. Both groups have attenuated flow reserve and a high prevalence of ischaemia. Impaired CFR predicts adverse cardiovascular outcomes and patients with sCMD and fCMD both have similar rates of adverse outcomes. Finally, stress perfusion cardiac magnetic resonance (CMR) imaging derived quantitative parameters, such as myocardial perfusion reserve index (MPRI), have been shown to correlate well with invasively measured CFR and adverse cardiovascular outcomes. Whilst their diagnostic utility

is being increasingly recognised, whether impaired CFR and MPRI predict response to anti-ischaemic therapy in patients with ANOCA is not known.

3 Study aim

To determine if invasive and non-invasive characterisation predicts response to anti-ischaemic therapy in patients with angina and non-obstructive coronary arteries (ANOCA).

4 Outcome measures

4.1 Primary outcome

Change in exercise time (in seconds) compared with baseline.

4.2 Major Secondary outcome

Change in Seattle Angina Questionnaire (SAQ) Summary Score compared with baseline.

4.3 Other outcome measures

4.3.1 Exercise treadmill test parameters

- Change in Time (s) to 0.1mV ST-depression compared with baseline
- Change in rate pressure product at 0.1mV ST depression compared with baseline
- Change in Maximum ST-segment deviation (mV) compared with baseline
- Change in Metabolic equivalent (METs) compared with baseline

4.3.2 Seattle Angina Questionnaire components

- Change in SAQ Component scores (Angina Limitation, Angina Stability, Angina Frequency, Treatment Satisfaction and Quality of Life) compared with baseline

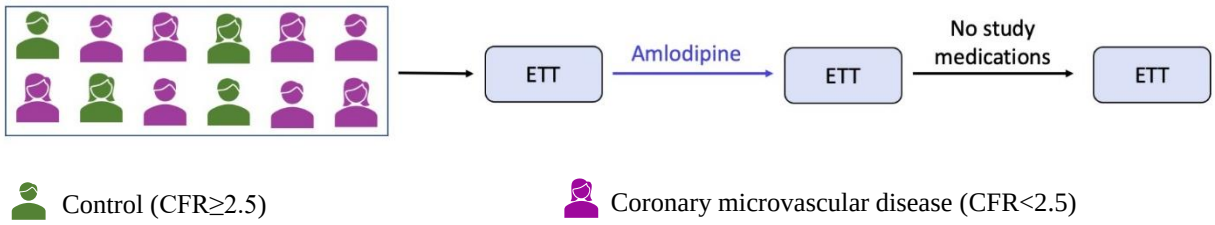
4.4 Exploratory outcomes

- Change in delta (pre- versus post- exercise) cardiac biomarkers compared with baseline
- Change in smartphone device derived stepcounts compared with baseline

5 Hypotheses and Sample size calculations

5.1 Hypothesis I_A:

Patients with ANOCA and coronary microvascular disease (CMD, defined by a CFR<2.5) will have a greater improvement in their exercise capacity in response to Amlodipine, compared to patients with ANOCA and normal microvascular function (controls, defined by a CFR≥2.5).

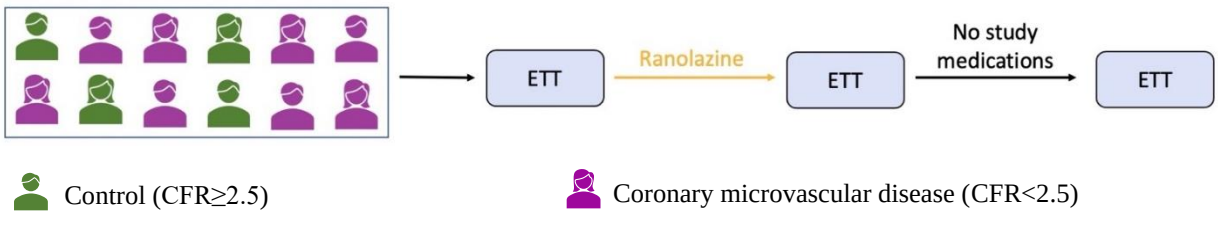


Assuming a 2:1 allocation ratio (as reported in the literature for patients with high pre-test probability of disease), 49 patients with CMD and 25 controls will provide 80% power (α=0.05) to detect a 60 second* difference in exercise time between the groups (standard deviation for change in exercise time is 85 seconds).

** The minimum clinically meaningful difference is considered 30 seconds.*

5.2 Hypothesis I_B:

Patients with ANOCA and coronary microvascular disease (CMD, defined by a CFR<2.5) will have a greater improvement in their exercise capacity in response to Ranolazine, compared to patients with ANOCA and normal microvascular function (controls, defined by a CFR≥2.5).



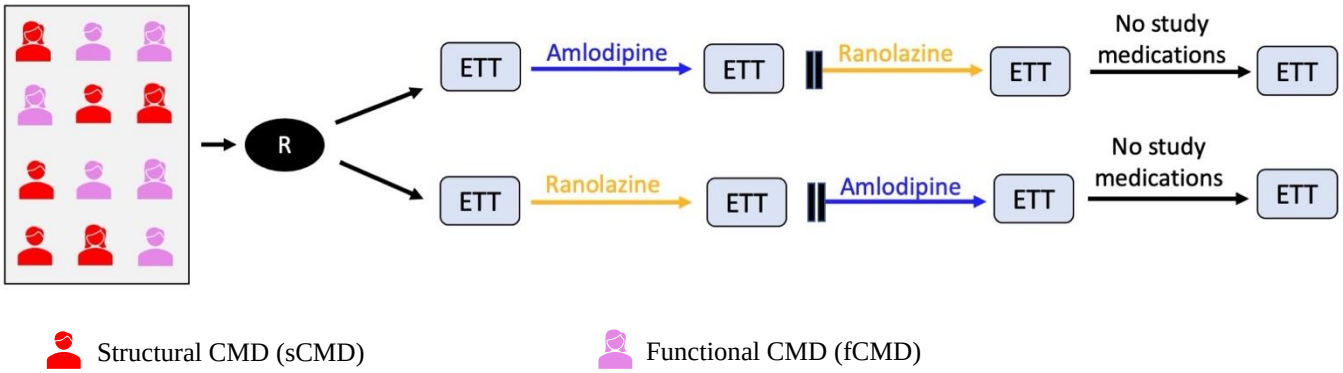
Power calculation as for hypothesis I_A.

5.3 Hypothesis II_A

Endotyping CMD will allow selection of more specific therapies than identifying CMD alone. We hypothesise that patients with *functional* coronary microvascular disease (fCMD, defined by a CFR<2.5 and hMR<2.5mmHg/cm/s) will have a greater improvement in their exercise capacity in response to ranolazine compared to amlodipine.

5.4 Hypothesis II_B

Patients with *structural* coronary microvascular disease (sCMD, defined by a CFR<2.5 and hMR≥2.5mmHg/cm/s) will have a greater improvement in their exercise capacity in response to amlodipine compared to ranolazine.



Assuming a 1:1 allocation ratio and a standard deviation for change in exercise time of 85 seconds, the sample sizes required to show the predicted differences is response to Amlodipine and Ranolazine at 80% power and 5% significance are detailed in the table below:

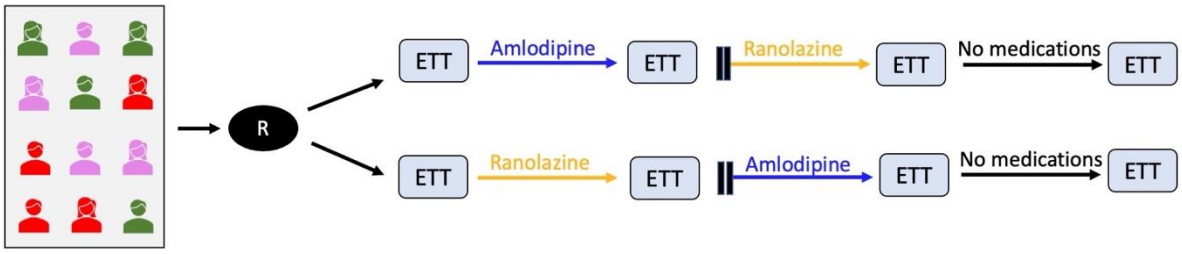
Difference in change in exercise time (between treatments), seconds	Sample size
60	18 patients with fCMD (Hyp II_A) and 18 with sCMD (Hyp II_B)
45	30 patients with fCMD (Hyp II_A) and 30 with sCMD (Hyp II_B)

Therefore, the sample size required to address hypothesis I (74 patients with approximately 50 patients expected to be classified as having CMD, comprising 25 patients with fCMD and 25 with sCMD) will facilitate detection of a difference in improvement of exercise time of at

least 60 seconds. If the final population enrolled includes higher numbers of fCMD and/or sCMD patients, this would provide additional power to detect even smaller differences in exercise time.

Carryover effects will be minimised by the inclusion of a 7-day washout period between the two anti-ischaemic therapies; however, we will carry out formal testing for carryover effects before assessing for treatment effects. The presence/absence of period (training) effects will be assessed by the change in exercise time at the 4th visit compared to the 1st visit.

To maintain **phenotype blinding** throughout, both experiments will be carried out in parallel to each other as part of a master study design (study flow chart below).



5.5 Cardiac magnetic resonance (CMR) imaging substudy

A myocardial perfusion reserve index (MPRI) of <2.2 on quantitative stress perfusion CMR identifies patients with an impaired CFR with an excellent diagnostic accuracy. We aim to assess whether characterisation by quantitative stress perfusion CMR predicts response to anti-ischaemic therapy in patients with ANOCA. A cohort of patients who have undergone paired coronary physiology and quantitative stress perfusion CMR assessment will be included in this substudy.

5.5.1 Hypothesis III$_A$

Patients with a *non-invasive diagnosis of CMD* (MPRI<2.2) will have greater improvement in their exercise capacity in response to amlodipine, than patients non-invasively classified as controls (MPRI≥2.2).

5.5.2 Hypothesis III_B

Patients with a *non-invasive diagnosis of CMD* (MPRI<2.2) will have greater improvement in their exercise capacity in response to ranolazine, than patients non-invasively classified as controls (MPRI≥2.2).

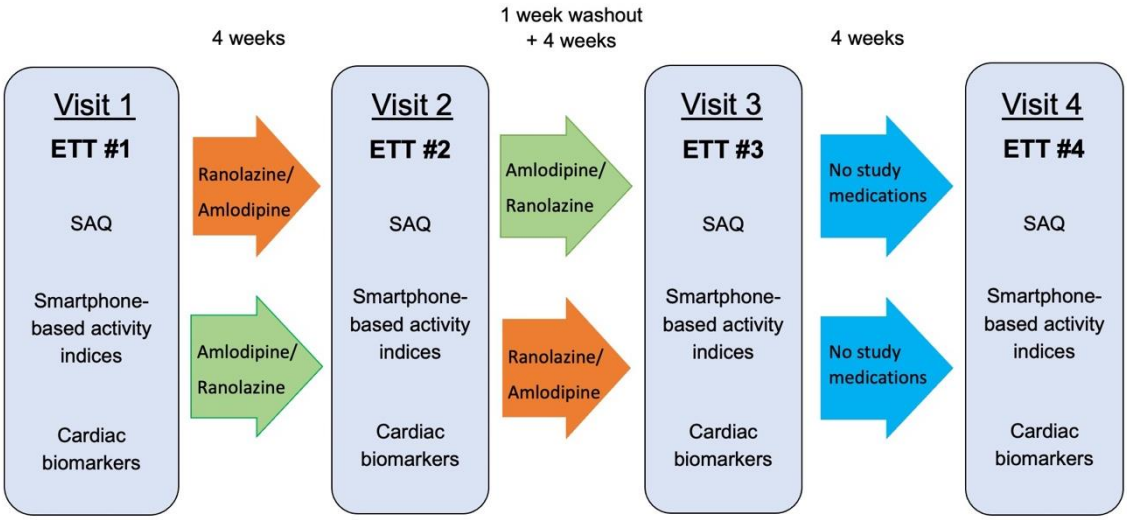
5.5.3 Exploratory comparison

The relative accuracy of invasive (CFR<2.5) and non-invasive (MPRI<2.2) CMD diagnoses at predicting an improvement in exercise time with a) Amlodipine and b) Ranolazine will be assessed by comparison of areas under the curve by ROC analysis using the Delong-Delong method

6 Study flow

ChaMP-CMD is a prospective randomised phenotype-blinded crossover trial designed to assess the utility of invasive and non-invasive measures of an ischaemic substrate at predicting response to anti-ischaemic therapy in patients with angina and non-obstructive coronary arteries.

This composite study design allows us to study distinct biologically plausible hypotheses in parallel, whilst maintaining phenotype-blinding.



7 Randomisation and Blinding

Patients and researchers will be blinded to patients' invasive and non-invasive physiology (CFR, hMR and MPRI) throughout the study. Hence, although patients are aware of which medication they will be taking at each stage of the study, given that they are blinded to their physiological phenotype, they will remain unaware of the expected efficacy of each medication in their particular case. Drug randomisation will be carried out during visit 1 using a simple randomisation model with 1:1 allocation (sequences being Amlodipine-Ranolazine and Ranolazine-Amlodipine). Given that this is a crossover trial, each patient serving as their own control, a block or stratified randomisation is not necessary.

8 Study populations

8.1 Subject inclusion criteria

- Typical exertional angina (CCS≥2)
- Unobstructed coronary arteries (fractional flow reserve >0.80)
- Normal left ventricular systolic function (LVEF >50%)

8.2 Subject exclusion criteria

- Patients who are unable or unwilling to consent
- Contraindications to adenosine
- Contraindications to Amlodipine and/or Ranolazine
- Patients who are already taking the study medications for clinical reasons and are unable to stop them
- Presence of more than moderate valve disease
- Previous percutaneous coronary intervention or bypass surgery
- Known structural heart disease (e.g. cardiomyopathy or congenital heart disease)
- Pregnant or breastfeeding females
- Patients who are unable to exercise on a treadmill or those who can exercise for >540seconds in the absence of any revealed cardiac symptoms on baseline exercise test

9 Definition of Populations for Analysis

All patients who provide informed consent will be accounted for in this study. A consort flow diagram will be produced to describe the passage of patients through the trial from enrolment, randomisation and analysis. Study withdrawals and major protocol violations will also be indicated.

For both experiments, the analysis will be reported on a per protocol basis to allow data on all patients to be included even when they were intolerant to one medication. An intention to treat analysis will also be carried out.

10 Statistical analyses

The hypotheses (I and II) will be tested in a hierarchical order, i.e. hypothesis I will be tested first and if the first null hypothesis is rejected, we will proceed to assess hypothesis II. Given the hierarchical hypothesis testing approach, no adjustment is planned of the significance level (alpha=0.05).

Any, post-hoc, exploratory analyses completed to support planned analyses, which were not identified in this SAP, will be documented and reported in the relevant trial reports. Any results from unplanned analyses will be clearly identified in the text of the trial reports.

Data analyses will be performed on GraphPad Prism version 9 and SPSS version 26 or later versions.

11 Methods for Handling Withdrawals and Missing Data

Patients who are randomised but withdraw before visit 2 will not be included in any further analyses. Patients who withdraw/cannot tolerate one drug but complete the other drug regimen will be included in the analysis pertaining to the completed drug. Patients with <80% medication adherence or those who are unable to attend for their exercise treadmill test within 1 week of the planned date will be excluded from analysis for the affected drug.

12 Analysis of outcome measures

The primary outcome measure will be analysed and reported as following.

1. Continuous variable (mean±SD change in exercise time (s) from baseline for both anti-ischaemic agents)

2. Binary variable (percentage of patients with Δexercise time >60s compared to baseline for both anti-ischaemic agents)

We plan to undertake a limited number of subgroup analyses for the primary outcome measure. Since the subgroup analyses are secondary analyses and exploratory in nature, the trial has not been powered for these. The pre-specified subgroup analyses will be performed on the following variables:

- Age
- Sex
- Diabetes
- Hypertension

13 Analysis and presentation of demographics and other baseline characteristics

13.1 General

Continuous variables will be described by the means and standard deviation (SD) except for skewed variables which may be described by the median and interquartile range (IQR). Categorical variables will be described by frequency and percentages in each category by treatment group.

13.2 Demographics

Demographic data collected at baseline include:

- Sex

- Ethnicity – Caucasian, Asian, African-Caribbean, Other
- Date of birth (and age)

13.3 Baseline Physical Examination

Patients will be examined at baseline and the following characteristics recorded:

- Height
- Weight
- Body mass index
- Blood pressure (systolic and diastolic)
- LVEF (echocardiograph: Simpson's biplane) %

13.4 Screening Medical History

The participants will have the following baseline disease specific characteristics recorded:

- Hypertension, diabetes mellitus, hyperlipidaemia, smoking history
- Vascular bed disease
- Angina typicality score (1-3)
- Angina status (CCS grade 0-4)
- NYHA class (1-4)
- Blood tests (Hb, Cr, lipids, NT-proBNP, HbA1C)

13.5 Medications

All cardiac medications being taken at randomisation will be summarised and continued for the duration of the study.