MUscle Side-Effects of atorvastatin in coronary patients (MUSE)

Statistical Analysis Plan

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STATISTICAL ANALYSIS PLAN for MUscle Side-Effects of atorvastatin in coronary patients (MUSE) – a randomized controlled trial

SIGNATURE PAGE

PRINCIPAL/COORDINATING INVESTIGATOR:

John Munkhaugen, MD, PhD
Department of Medicine
Drammen Hospital
Vestre Viken Trust

3/12-19

John Munkhaugen

(e-sign)

Signature

Date (dd/mmm/yyyy)

TRIAL STATISTICIAN:

Morten W Fagerland, PhD
Oslo Centre for Biostatistics and Epidemiology
Oslo University Hospital

3/12-19

Morten Wang Fagerland

(e-sign)

Signature

Date (dd/mmm/yyyy)

ABBREVIATIONS

AE	Adverse Event
ATC	Anatomical/Therapeutic/Chemical
CI	Confidence Interval
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
MedDRA	Medical Dictionary for Regulatory Activities
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
SOC	System Organ Class
ATV	Atorvastatin
SAMS	Statin associated muscle symptoms
CHD	Coronary heart disease

STATISTICAL ANALYSIS PLAN for MUscle Side-Effects of atorvastatin in coronary patients (MUSE) – a randomized controlled trial

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STATISTICAL ANALYSIS PLAN for MUscle Side-Effects of atorvastatin in coronary patients (MUSE) – a randomized controlled trial

1 Introduction

1.1 Background and rationale

Statin non-adherence (i.e. patients not taking their prescribed drugs) remains a major public health concern in cardiovascular disease patients, leading to adverse outcomes in terms of morbidity, mortality and healthcare costs[1-3]. The principal reason for statin non-adherence and discontinuation is statin-associated muscle symptoms (SAMS)[4]. SAMS, comprising a heterogeneous group of muscle symptoms including pain/aching, stiffness, tenderness or cramps, usually with normal or minimally elevated creatinine kinase levels, is prevalent challenge in clinical practice[4]. In contrast, serious statin side-effects, including liver failure, rhabdomyolysis, or myositis with elevated creatinine kinase levels in blood, are very rare[4,5]. In randomized trials, statin therapy appears to cause only a slightly increased risk of side-effects of 1-5% compared with placebo, far below the prevalence reported in clinical practice, amounting to 30% in observational studies[6,7]. Strict entry criteria in the randomized statin trials, excluding patients with polypharmacy, multiple comorbidities, elderly, females and low body weight, factors that predispose to musculoskeletal symptoms, may in part explain the diverging frequency of SAMS[4]. A major limitation of observational studies is a lack of blinding. Patients on statins may expect to experience side-effects, and therefore report a higher percentage than in a comparable population not on statins, the so-called 'nocebo' effect. Two previous studies, in subjects without cardiovascular disease, has tested whether SAMS reported by the patients are related the statin therapy[8-9]. In a randomized, double-blind crossover study that included patients complaining of SAMS at study start, only 36% experienced that their muscle symptoms persisted during treatment with simvastatin 20 mg and disappeared during placebo treatment[8]. Accordingly, SAMS was confirmed to be related to the statin treatment in one-third of the patients. The proportion of CHD patients with confirmed SAMS, treated with potent statins recommended for these patients, remains unknown. Furthermore, the clinical presentation (i.e. location, intensity and characteristics) of the muscle symptoms in CHD patients with and without confirmed SAMS remains to be investigated.

Although several mechanisms have been proposed, it remains unclear how statins produce muscle symptoms, and reliable diagnostic biomarkers for the prediction or diagnosis of SAMS are lacking[4,5]. For patient follow-up it is highly relevant to identify markers that predict the occurrence of muscle symptoms, and thus enable preventive actions. Individual variations in statin pharmacokinetics, mitochondrial dysfunction or coenzyme Q10 deficiency are suggested mechanisms for SAMS[4,5]. A few observation studies indicate that pharmacokinetic alterations in statin metabolites may contribute to SAMS[10,11]. The lactone metabolites of statins seem to be most potent in inducing myotoxic effects compared to the corresponding acid metabolites[10]. Accordingly, plasma concentrations of atorvastatin lactones have been associated with clinical muscle symptoms[11]. In the present project we will determine the relationship between muscle symptoms and blood levels of atorvastatin metabolites using our liquid chromatography mass spectrometry method[12]. Importantly, we have pilot data indicating significantly higher levels of atorvastatin lactone metabolites (sufficient for statistical significance) in patients reporting subjective SAMS, compared with those not reporting muscle symptoms[13]. If statin metabolite levels prove to be significantly stronger correlated with subjective muscle symptoms in patients with confirmed SAMS compared to those without in a randomized placebo-controlled study, this may pave the way for diagnostic testing of confirmed SAMS.

1.2 Trial Objectives

1.2.1 Primary Objective

To estimate the effect of atorvastatin on muscular symptom intensity in coronary patients with subjective SAMS.

1.2.2 Secondary Objectives

The secondary objectives of this study are:

- To determine the proportion of patients who report muscle symptoms on atorvastatin treatment compared with on placebo (dichotomous SAMS classification)
- To determine the correlation between muscular symptom intensity and blood concentrations of parent drug and metabolites of atorvastatin in patients with confirmed SAMS*
- To determine the diagnostic properties of blood concentrations of parent drug and metabolites of atorvastatin for classification of confirmed SAMS*
- To determine adherence to the study medication (i.e. atorvastatin and placebo) during the treatment periods
- To compare blood concentrations of parent drug and the metabolites of atorvastatin between patients with failing placebo-test (i.e. non-SAMS)* for connecting SAMS to atorvastatin and the control group without muscle symptoms
- To describe study safety

*****Confirmed SAMS*** *is defined as at least ≥25% higher mean muscle symptom intensity (measured on a 0-10 cm Visual Analogue Scale [VAS])* ***and*** *a 1cm difference during the last 3 weeks on treatment with atorvastatin compared to placebo. All other patients in the are defined as non-SAMS.*

1.2.3 Exploratory Objectives

The exploratory objective of this study is:

- To compare patients with and without confirmed SAMS regarding muscle symptom characteristics and location
- To study sociodemographic, clinical, and psychosocial characteristics (PROMS and clinical data) in patients with and without confirmed SAMS

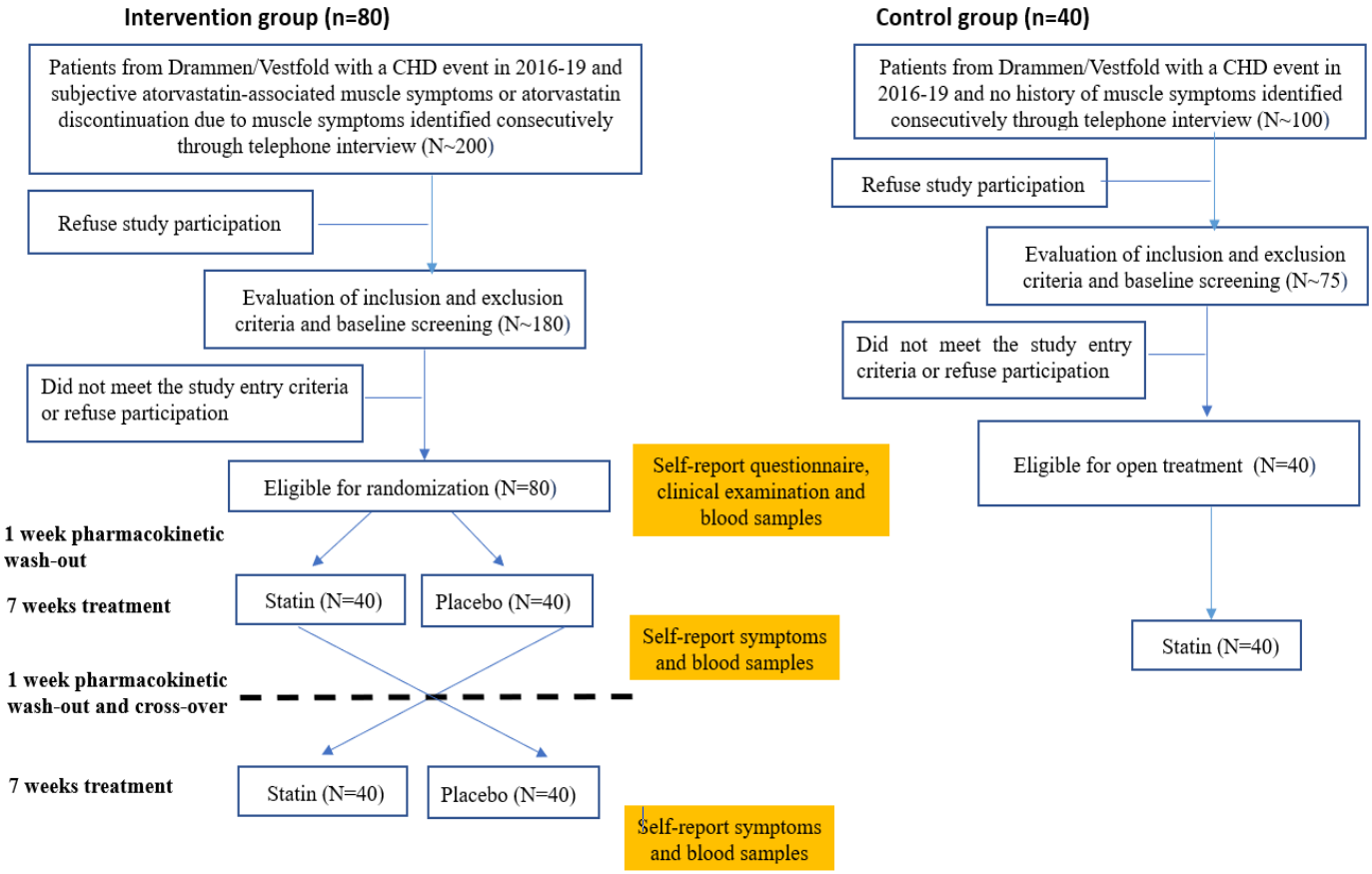
2 Trial Methods

2.1 Trial Design

MUSE is a randomized, double-blinded, multi-center, cross-over phase 4 study in Norway that aimed to include 80 CHD patients with subjective SAMS during atorvastatin therapy who report i) ongoing SAMS or ii) atorvastatin discontinuation because of SAMS. The patients will be randomized 1:1 to either atorvastatin in the first period and placebo in the second period or placebo in the first period and atorvastatin in the second period, in an AB/BA cross-over design. (Figure 1) In addition, 40 coronary patients on atorvastatin (at least 40 mg) but without SAMS at the time of inclusion in the

study will be included consecutively and undergo an open 7 weeks treatment period with atorvastatin in a similar dose as in those with subjective SAMS.

Figure 1. Study flow-chart



2.2 Randomisation

Eligible patients will be randomized to double-blinded prescription of atorvastatin or a matched placebo tablet in a 1:1 ratio using the electronic randomisation system (Viedoc™) of the Clinical Trials Unit (CTU) at Oslo University Hospital. Block randomization with block size 4 and 6 in random order, stratified according to centre (Drammen Hospital and Hospital of Vestfold) and previous atorvastatin discontinuation (yes/no), will be used.

The randomization process is described in full within the clinical trial protocol. Details of the randomization including the final random allocation list are held securely and unavailable to unauthorized trial personnel.

2.3 Sample size

Sample size calculations are based on our being able to detect a 1 cm difference in the VAS symptom score between the treatment periods on atorvastatin and placebo since the smallest change in VAS symptom score corresponding to 'a little more' or 'a little less' symptoms was 1.3 cm, with a lower limit of the CI at 1 cm in a previous study by Gallagher et al[14]. Gallagher et al. report a standard

deviation (SD) of 1.7 for a difference of 1.0 between two VAS symptom scores. Because of the differences in both populations and specific VAS scale between that study in this one, we use SD=2.5 to account for a much larger variation in this study. With n=68, we will have 90% power to detect a difference of 1.0, using a one-sample T test. With n=68, we will also have 80% power to detect a difference of 40% SAMS under statins vs. 15% SAMS under placebo, using the McNemar test for paired probabilities. To account for some missing information due to drop-outs or and protocol deviations, we plan to include 80 patients.

2.4 Statistical Framework

2.4.1 Hypothesis Test

This study is designed to detect that the reported SAMS under atorvastatin treatment is higher (corresponding to at least "a **little more***" symptoms) than under placebo treatment.

- The primary null hypothesis is that the level of reported SAMS is equal under atorvastatin and placebo treatment.
- The primary alternative hypothesis is that there is a difference in the level of reported SAMS between atorvastatin and placebo treatment.

There is only one identified primary analysis in this trial. All other efficacy analyses will be regarded as supportive or exploratory.

* *See Section 2.3*

2.4.2 Decision Rule

This trial is designed to address a single primary outcome. Higher SAMS under either atorvastatin treatment or placebo treatment is claimed if the primary null hypothesis is rejected at the 5% (alpha = 0.05) significance level (two-sided).

2.5 Statistical Interim Analyses and Stopping Guidance

There will be no interim analyses in this trial.

2.6 Timing of Final Analysis

The main analysis is planned when all patients have concluded 16 weeks of treatment, all data up to 16 weeks have been entered, verified and validated and the primary database has been locked.

2.7 Timing of Outcome Assessments

For all clinically planned measures, visits should occur within a window of the scheduled visit. Visits outside visit window is regarded a protocol deviation. The target day and visits window are defined in the protocol as:

Visit Label	Target Day	Definition (Day window)
Screening	Day -60 to -1	Prior to Day 0
V1. Baseline	Day 0 (Randomization)	Day 0
V2	56**	Target day ± 2 days
V3	112**	Target day ± 2 days
Last study visit*	112**	112

**The last study visit is defined as the visit following the last visit with randomised treatment, and where there is a study end statement.*

***According to the protocol, V2 and V3 (last visit) may be scheduled after a treatment period of at least three consecutive study weeks (21 days) in participants with intolerable muscle complaints.*

For analysis and tabulation purposes, we define study time points as

Time Point Label	Target Day	Definition (Day window)
TP1. Baseline	Day 0 (Randomisation)	Information up to randomisation
TP2. Week 8	56	Days 1 to 56
TP3. Week 16	112	Days 56 to 112

If more than one visit fall into the same time point interval, information on all visits will be used in the analyses.

3 Statistical Principles

3.1 Confidence Intervals and P-values

All calculated P-values will be two-sided and compared to a 5% significance level. If a P-value is less than 0.05, the corresponding treatment difference will be denoted as statistically significant. All efficacy estimates will be presented with two-sided 95% confidence intervals. As there is only one primary null hypothesis to be tested in this trial, there will be no adjustments for multiplicity.

3.2 Adherence and Protocol Deviations

3.2.1 Adherence to Allocated Treatment

Adherence to study treatment is based on both **indirect** and **direct** methods:

Indirect method:

Adherence for each patient is based on pill counts in returned containers. It is defined as:

% adherence = proportion of days covered (PDC) = (1 – number of remaining pills/number of pills delivered to patient) x 100%

Low adherence = PDC <80% in at least one of the two treatment periods.

Mean (SD) PDC and the proportion of patients with PDC <80% will be presented for all patients and stratified for (i) treatment order allocation and (ii) patients with confirmed SAMS vs. non-SAMS.

Direct method:

Adherence to study treatment will also be determined directly in blood at the end of each treatment period with our liquid chromatography–tandem mass spectrometry method[12] classified according to the algorithm published by Kristiansen et al.[15].

Adherence = patients being classified as non-adherent to atorvastatin in the end of the placebo study period *and* adherent to atorvastatin therapy in the end of the atorvastatin study period according to Kristiansen et al.[15].

Low adherence = all patients not classified as adherent as defined above.

3.2.2 Protocol Deviations

The following are pre-defined major protocol deviations regarded to affect the efficacy of the intervention:

- Entering the trial when the eligibility criteria should have prevented trial entry
- Discontinuation of intervention before 3 weeks in any treatment period
- Major change in concomitant lipid lowering medication reported by the patients
- Use of prohibited rescue medication
- Received or used other intervention than allocated

Protocol deviations are classified prior to unblinding of treatment. The number (and percentage) of patients with major and minor protocol deviations will be summarised with details of type of deviation provided.

3.3 Analysis Populations

The Enrolled set will include all patients who have provided informed consent and have been included into the study data base.

The Full Analysis Set (FAS) will be defined as all patients randomly assigned to a treatment group having completed at least three consecutive study weeks on each treatment period.

The Safety Analysis Set will include all patients having received at least one study treatment tablet after randomisation.

The Per Protocol Analysis Set (PPS) will include all randomised patients meeting the study eligibility criteria and with no major protocol deviations affecting the treatment efficacy (i.e. <10% missing data* in the patient diary and >80% tablet adherence in the drug and placebo period, separately), and also being classified as non-adherent to atorvastatin in the end of the placebo study period by direct blood

sample measurement according to Kristiansen et al.[15]. Patients being classified as partial adherent or non-adherent to atorvastatin therapy in the end of the atorvastatin study period by direct blood sample measurement (Kristiansen et al.[15]) will be excluded from the data analyses concerning blood levels of parent drug and metabolites.

**The percentage of missing data for the core self-reported variables in the diary (i.e. the VAS 0-10 cm likert scale and the 0-10 numeric rating scale) will be reported.*

4 Trial Population

4.1 Screening Data, Eligibility and Recruitment

The total number of screened patients and reasons for not entering the trial will be summarised and presented. A CONSORT flow diagram (appendix A) will be used to summarise the number of patients who were:

- assessed for eligibility at screening
- eligible at screening
- ineligible at screening*
- eligible and randomised
- eligible but not randomised*
- received the randomised allocation
- did not receive the randomised allocation*
- lost to follow-up*
- completed intervention and assessments
- completed assessments but not intervention
- withdrew consent*
- discontinued the intervention*
- randomised and included in the primary analysis
- randomised and excluded from the primary analysis*

*reasons will be provided.

4.2 Baseline Patient Characteristics

Baseline characteristics of intervention group stratified by treatment allocation (Atorvastatin-Placebo vs. Placebo-Atorvastatin) will be tabulated, using descriptive statistics for continuous variables, and number and percentages of patients for categorical variables. There will be no statistical analysis of treatment difference. Any clinical important imbalance between the treatment groups will be noted.

The patient demographics and baseline characteristics to be summarised include study center (Drammen/Vestfold), previous atorvastatin discontinuation (yes/no), age in years, gender, education (high, low), living alone (yes/no) lipid lowering treatment (yes/no, type, high-intensity therapy, low-intensity therapy, total number of statins used), coronary index diagnosis (myocardial infarction vs. stable/unstable CHD), >1 coronary event prior to the index event, comorbidities (heart failure, hypertension, diabetes, stroke/TIA, rheumatic or inflammatory disease, arthrosis, hypo or

hyperthyroidism), concomitant medication used regularly (total number of drugs, potentially interacting drugs, and analgesics, coronary risk factors (LDL-C, smoking status, physical activity, BMI, hsCRP), other laboratory tests (estimated GFR, creatinine kinase, alanine aminotransferase), consulted primary care physician regarding statin side-effects (yes/no).

5 Outcome Definitions

5.1 General Definitions and Derived Variables

5.1.1 Muscular symptom intensity

Individual mean difference in muscular symptom intensity = Mean muscular symptom intensity (regardless of characteristics and location) reported by the patient the last three weeks during the treatment period on atorvastatin – the last three weeks during the treatment period on placebo. The last consecutive three weeks completed during each treatment period will be used in patients who discontinue due to intolerable muscle symptoms.

Muscular symptom intensity is reported through a 0-10 cm Visual Analogue Scale (VAS) in a patient diary delivered at study start. The patients are asked to also report muscular symptom intensity on a 0-10 Likert scale. Results from the VAS scale will be replaced by the results from the Likert scale in case of missing data on the VAS scale.

VAS scores over the last 3 weeks in each treatment period have been chosen as primary end-point for three reasons: i) to ensure steady state concentrations of atorvastatin; ii) to maximize the likelihood for the symptoms reported to being truly related to the current (and not previous) treatment period; iii) the duration of the treatment period being sufficiently long to produce SAMS (>3 weeks). VAS score has also been chosen as primary outcome in two previous SAMS studies[16,17].

5.1.2 The proportion of patients who report muscle symptoms on atorvastatin treatment compared with on placebo (dichotomous SAMS classification)

The proportion of patients who report muscle symptoms on atorvastatin treatment and not on placebo (dichotomous SAMS classification). A 25% intra-individual change in the VAS symptom score has been regarded clinically relevant in a previous validation study of the scale[14]. As previously described it has been reported as the smallest change in VAS symptom score corresponding to ‘a little more’ or ‘a little less’ symptoms was 1.3 cm, with a lower limit of the CI at 1.0 cm in a previous study by Gallagher et al[14] Accordingly, confirmed SAMS will be defined as a 25% higher individual VAS score during the treatment period on atorvastatin vs. placebo and >= 1.0 cm absolute difference.

5.1.3 Atorvastatin and metabolites level in blood

In the MUSE study design paper[13], we have described that “atorvastatin and metabolites levels in white blood cells (i.e. lymphocytes) is also a relevant candidate marker for SAMS in addition to plasma”. Importantly, only plasma levels of atorvastatin and metabolites will be determined and related to SAMS in the main study. A separate paper will determine the relationship between SAMS and statin and metabolites level in lymphocytes.

Table 1 shows an overview of the different combinations of parent drug (atorvastatin) and metabolites that will be related to SAMS in the study. We expect the variables in bold to be the ones most likely to be related to SAMS.

Table 1. Parent drug and metabolites of ATV to be determined.

Variables	Abbreviations
Atorvastatin acid C0	AA C0
2-OH-atorvastatin acid C0	2OH-AA C0
4-OH-atorvastatin acid C0	4OH-AA C0
Atorvastatin lactone C0	AL C0
2-OH-atorvastatin lactone C0	2OH-AL C0
4-OH-atorvastatin lactone C0	4OH-AL C0
Sum acids C0	Sum A C0
Sum lactones C0	Sum L C0
Sum acids and lactones C0	Sum A+L C0
Atorvastatin acylglucuronide C0	AAG C0
Atorvastatin acid C2	AA C2
2-OH-atorvastatin acid C2	2OH-AA C2
4-OH-atorvastatin acid C2	4OH-AA C2
Atorvastatin lactone C2	AL C2
2-OH-atorvastatin lactone C2	2OH-AL C2
4-OH-atorvastatin lactone C2	4OH-AL C2
Sum acids C2	Sum A C2
Sum lactones C2	Sum L C2
Sum acids and lactones C2	Sum A+L C2
Atorvastatin acylglucuronide C2	AAG C2

5.1.4 Muscular symptom location

Muscular symptom location is obtained through patient self-report measured with the Brief Pain Inventory at the end of each 7-weeks treatment period. Presence of symptoms (yes/no) are noted on the following locations: neck/shoulder/trunk, proximal upper extremities, proximal lower extremities, distal upper extremities, distal lower extremities and bilateral vs. unilateral.

Detailed differences in muscle symptom location (i.e. «Dermatome pain map» (Margoli Dermatome Pain Map) Margolis RB et. Al. Pain 24:57–65, 1986) will be explored in a separate sub-study publication.

5.1.5 Muscular symptom characteristics

Muscle symptom characteristics are obtained through patient self-report measured with McGill Pain Questionnaire administrated at the end of each 7-weeks treatment period. Fifteen different characteristics are rated from none (0 points) to strong (3 points).

5.2 Primary Outcome Definition

The primary outcome is the difference between the VAS symptom scores for the mean of the last three weeks of the atorvastatin period and the mean of the last three weeks of the placebo period.

5.3 Secondary Outcomes Definitions

5.3.1 Dichotomous SAMS classification

The proportion of patients with confirmed SAMS, as defined in Section 5.1.2, where the mean of the symptoms scores in the last three weeks of each treatment period is used.

5.3.2 Relationship between muscle symptom intensity and atorvastatin and metabolites level in blood

The correlation between individual differences in mean muscular symptom intensity and atorvastatin and metabolites in blood plasma among patients with confirmed SAMS, as defined in Section 5.1.2. The 20 variables in Table 1 will be used.

5.3.3 Diagnostic properties of atorvastatin and metabolites for classification of confirmed SAMS

Sensitivity, specificity, and area under the ROC curve of blood concentrations of parent drug and the metabolites of atorvastatin for the classification of confirmed SAMS, as defined in Section 5.1.2 The 20 variables in Table 1 will be used.

5.3.4 Statin (ATV) adherence

Statin adherence determined with pill counts in returned tablet boxes and atorvastatin plus metabolites concentration in blood, as defined in Section 3.2.1.

5.3.5 Atorvastatin and metabolites level in patients with failing placebo-test for connecting SAMS to atorvastatin and the control group without muscle symptoms

Difference between levels of atorvastatin and its metabolites in blood plasma at the end of the treatment period with atorvastatin for patients with failing placebo-test for connecting SAMS to atorvastatin (i.e. non-SAMS) and the control group without muscle symptoms. The 20 variables in Table 1 will be used.

5.3.6 Study safety

- Proportion of patients with new-onset CHD symptoms (e.g. angina, dyspnoea)
- Intolerable muscle symptoms leading to discontinuation from the treatment arm
- Proportion of patients with creatine kinase (CK) > 10 times upper limit of the normal range or alaninaminotransferase (ALT) > 3 times upper limit of the normal range in blood

- Proportion of patients with serious adverse events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARS).

5.3.7 Muscle symptom location

We define an outcome that is the difference between the presence (yes=1, no=0) of muscle symptoms during atorvastatin treatment and the presence (yes=1, no=0) of muscle symptoms during placebo treatment for each of the following locations:

- neck/shoulder/ trunk
- proximal upper extremities
- proximal lower extremities
- distal upper extremities
- distal lower extremities
- Bilateral vs. unilateral

The outcomes (differences) have three possible values:

- -1, indicating that there are more symptoms on placebo
- 0, indicating that there are equal symptoms on atorvastatin and placebo
- 1, indicating that there are more symptoms on atorvastatin

In addition, we define an outcome that is the difference between the total number of muscle locations marked during atorvastatin treatment and the total number of muscle locations marked during placebo treatment.

5.3.8 Muscle symptom characteristics

We define an outcome that is the difference between the rating (scale = 0,1,2,3) of muscle symptom characteristics during atorvastatin treatment and the rating (scale = 0,1,2,3) of muscle symptom characteristics during placebo treatment for each of the 15 characteristics. The outcomes (differences) have seven possible values: -3, -2, -1, 0, 1, 2, 3. Negative values indicate stronger characteristics on placebo, whereas positive values indicate stronger characteristics on atorvastatin.

5.3.9 Time to symptoms

The number of weeks from start of atorvastatin treatment to confirmed SAMS, as defined in Section 5.1.2, for the patients with confirmed SAMS.

The number of weeks from start of atorvastatin treatment to the week with the highest VAS symptom score (all patients).

5.3.10 Pharmacogenetics

The presence of the following genetic variables will be determined:

- SLCO1B1: CC genotype vs. CT genotype vs. TT genotype
- CYP3A5: *3/*3 genotype vs. (*1/*3 or *1/*1 genotype)
- CYP3A4: (*1/*22 or *22/*22 genotype) vs. *1/*1 genotype

6 Statistical Analysis

6.1 Carry-Over Effect

The length of each treatment period was chosen on the basis of previous evidence from a large case control study[18] and an observational study[17] suggesting that SAMS appear on median two and four weeks after re-challenge and initiation of statin treatment, respectively. The washout periods equate to more than 10 half-lives of atorvastatin[19] and the primary outcome will be analysed on the basis of symptom intensity in the last three weeks of each treatment period. Because SAMS improve after a median of two weeks following treatment discontinuation[18], we assume that there is no carry-over effect in this trial.

6.2 Period Effect

Because of the short treatment periods in this trial, it is very unlikely that there will be a trend over time that affects the trial or its patients; thus, we assume that there is no period effect in this trial.

6.3 Primary Outcome

The primary outcome, the difference between the VAS symptom scores for atorvastatin and placebo, will be estimated as the predictive overall margin of a linear regression model with the difference (atorvastatin minus placebo) as the dependent variable and the stratification factors in the randomization (i.e. centre and previous statin discontinuation) as covariates. The estimate and its 95% confidence interval will be presented, with a P-value for the null hypothesis that the difference is zero.

The primary analysis will be performed on the full analysis set. A secondary analysis will be performed on the per protocol set.

6.4 Secondary Outcomes

All secondary outcomes will be analysed on the FAS, except where noted otherwise.

6.4.1 Dichotomous SAMS classification

The proportion of patients with confirmed SAMS will be estimated as the number of patients with confirmed SAMS divided by the total number of patients in the full analysis set. A 95% confidence interval for the proportion will be estimated with the Wilson score confidence interval[20].

6.4.2 Relationship between muscle symptom intensity and atorvastatin and metabolites level in blood

The correlations between differences in muscular symptom intensity and levels of atorvastatin and metabolites among patients with confirmed SAMS will be estimated with Spearman rank correlation coefficients, with 95% confidence intervals estimated by the Bonett-Wright approximation[21]. In addition, scatter plots and linear regression analyses may be used to illustrate the associations.

6.4.3 Diagnostic properties of atorvastatin and metabolites for classification of confirmed SAMS

Methods for analysis of ROC curves and measures of diagnostic accuracy will be used to identify cut-off values of metabolite concentrations that can discriminate between confirmed SAMS and non-SAMS. These methods include calculating sensitivity, specificity, and area under the ROC curve (with 95% confidence intervals).

6.4.4 Statin adherence

Statin adherence will be presented with descriptive statistics.

6.4.5 Atorvastatin and metabolites level in patients with failing placebo test for connecting SAMS to atorvastatin and the control group without muscle symptoms

The comparison of levels of atorvastatin and its metabolites between the patients with failing placebo test (non-SAMS) and the patients in the control group (see Figure 1, the study flow chart) will be done with two-sample T tests with adjustment for unequal variances. The mean differences with 95% confidence intervals (based on the t-distribution) and the P-values from the tests will be presented.

6.4.6 Study safety

Study safety will be presented with descriptive statistics.

6.4.7 Muscle symptom location

The difference between atorvastatin and placebo in presence of symptoms locations will be compared between patients with confirmed SAMS (see definition in Section 5.1.2) and non-SAMS patients with the score test for effect in a proportional odds model[22] (the Wilcoxon-Mann-Whitney test). The number and percentages of patients in each of the three possible outcome categories (-1, 0, 1) will be presented by SAMS status, together with the P-value from the test.

6.4.8 Muscle symptom characteristics

The difference between atorvastatin and placebo in muscle symptom characteristics will be compared between patients with confirmed SAMS (see definition in Section 5.1.2) and non-SAMS patients with the score test for effect in a proportional odds model[22] (the Wilcoxon-Mann-Whitney test). The number and percentages of patients in each of the seven possible outcome categories (-3, -2, -1, 0, 1, 2, 3) will be presented by SAMS status, together with the P-value from the test.

6.4.9 Time to symptoms

Time to symptoms will be presented with descriptive statistics.

6.4.10 Pharmacogenetics

(i) The mean value of the atorvastatin exposure variable with the highest area under the ROC curve (from the analyses in Section 6.4.3) will be compared between the different genotypes in the genetic variables SLCO1B1, CYP3A5, and CYP3A4 (see Section 5.3.11). The mean (standard deviation) for each genotype will be presented, with a P-value for a test of the null hypothesis of equal means. The two-sample T test with adjustment for unequal variances will be used for CYP3A5 and CYP3A4 (two genotypes) and ANOVA will be used for SLCO1B1 (three genotypes).

(ii) The frequency of SLCO1B1, CYP3A4, and CYP3A5 genotypes will be compared among patients with confirmed SAMS (see definition in Section 5.1.2), non-SAMS and controls (see Figure 1, the study flow chart) with a Pearson chi-squared mid-P test for unordered r x c tables[21]. The comparisons for CYP3A4/5 will only be performed if there is a statistically significant difference between the genotypes in the atorvastatin exposure variable in (i).

6.5 Assumption Checks and Alternative Analyses

The assumption of normally distributed residuals in linear regression and for the two-sample T test will be checked by plotting histograms of the residuals and examining their descriptive statistics, such as mean, median, standard deviation, and coefficient of skewness.

In cases where the distribution of the residuals is deemed to deviate too much from the normal distribution to allow linear regression to be used, a median regression (i.e. a quantile regression) will be performed instead of the linear regression. Then, the median (with a 95% confidence interval and a P-value for the null hypothesis that the median is zero) will be reported instead of the mean. Similarly, if the two-sample T test is deemed to be unsuitable due to too large deviations from the normal distribution, median regression will be used, and differences in medians (with 95% confidence intervals) will be estimated instead of differences in means.

6.6 Missing Data

Due to very close monitoring of adherence to the protocol during the treatment periods, we expect few missing data for the primary outcome and other core self-reported measures. Missing values of VAS-scores in the diary will be replaced by corresponding values from the 1-10 likert scale (Numeric Rating Scale) that were reported simultaneously.

In the unlikely case that there are patients with missing data on both the VAS and NRS scales, those patients will be excluded from the main analyses, but included in a secondary sensitivity analysis (see next section).

6.7 Sensitivity Analyses

- The primary outcome will be analyzed on the per protocol set
- Missing data on the primary outcome will be imputed such that the patient with missing data will have no difference between the two treatment periods in the missing outcome.

6.8 Subgroup Analyses

There will be no subgroup analyses; however, some of the secondary outcomes are defined in subsets of the trial sample, or involve comparisons of groups defined by the SAMS classification:

- The analyses in Section 6.4.6 only include non-SAMS patients (and patients in the control group)
- The analyses in Section 6.4.8 compare patients with confirmed SAMS with non-SAMS patients
- The analyses in Section 6.4.9 compare patients with confirmed SAMS with non-SAMS patients
- The analysis of time to confirmed SAMS in Section 6.4.10 only includes patients with confirmed SAMS

7 Safety Analyses

Safety endpoints will be under the responsibility of the primary investigators at the participating centres and will be collected:

- Every 7th days: direct telephone contact with the patient for assessment of intolerable muscle symptoms and symptoms of unstable CHD (i.e. new-onset angina pectoris and/or dyspnoea) after a standardized protocol by a specially trained study nurse.
- Blood samples collected for analyses of ALAT and CK at the end of each 7 weeks treatment period or if intolerable muscle symptoms were reported by the patients.
- Continuous surveillance of serious adverse events (SAEs) obtained through direct weekly telephone contact with the patients and through continuous monitoring of hospital admissions during the study period.

7.1 Adverse Events

As outlined in Section 6.4.7, adverse events will be presented with descriptive statistics.

7.2 Clinical Laboratory Parameters

Safety (alanine aminotransferase, creatinine kinase) and clinical laboratory (estimated glomerular filtration rate, LDL-C, total-c, hsCRP, albumin, pharmacogenetics) parameters were collected and assessed (myoglobin was not assayed as creatinine kinase was considered sufficient).

7.3 Vital Signs

Changes in vital signs have not been monitored in the present study.

8 Statistical Software

The statistical analyses will be done in Stata/SE 16.0 (StataCorp LLC, College Station, TX) and Matlab R2014a (The MathWorks, Inc.).

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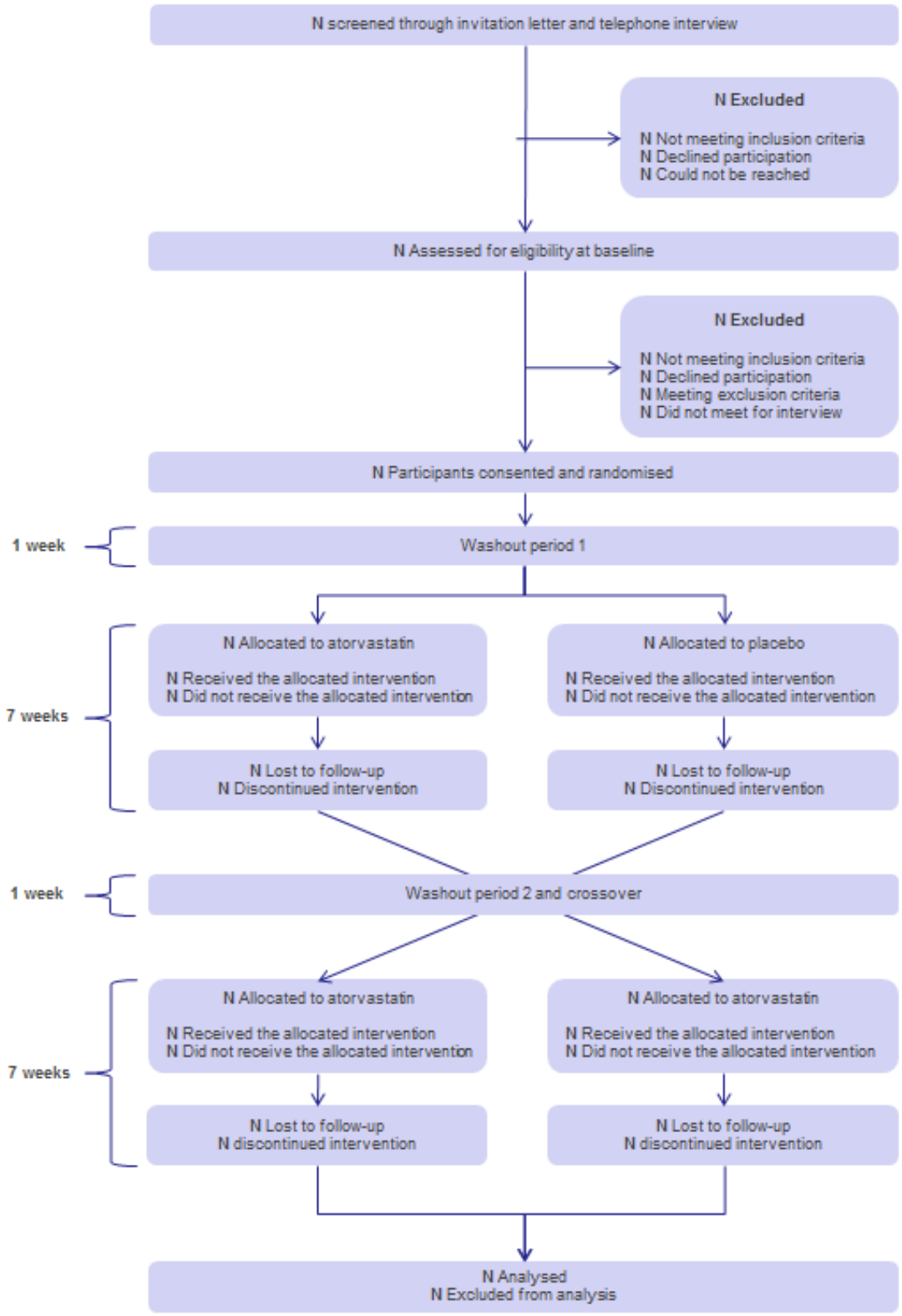
Appendix A: Consort flow diagram for cross-over trials


N screened through invitation letter and telephone interview
N Excluded
N Not meeting inclusion criteria
N Declined participation
N Could not be reached
N Assessed for eligibility at baseline
N Excluded
N Not meeting inclusion criteria
N Declined participation
N Meeting exclusion criteria
N Did not meet for interview
N Participants consented and randomised
1 week
Washout period 1
7 weeks
N Allocated to atorvastatin
N Received the allocated intervention
N Did not receive the allocated intervention
N Allocated to placebo
N Received the allocated intervention
N Did not receive the allocated intervention
N Lost to follow-up
N Discontinued intervention
N Lost to follow-up
N Discontinued intervention
1 week
Washout period 2 and crossover
7 weeks
N Allocated to atorvastatin
N Received the allocated intervention
N Did not receive the allocated intervention
N Allocated to atorvastatin
N Received the allocated intervention
N Did not receive the allocated intervention
N Lost to follow-up
N discontinued intervention
N Lost to follow-up
N discontinued intervention
N Analysed
N Excluded from analysis