Atorvastatin treatment in patients with coronary heart disease – adherence and muscle side effects

Oscar Kristiansen

Thesis for the degree of Philosophiae Doctor

Department of Medicine, Drammen Hospital, Vestre Viken Trust
and
Institute of Basic Medical Sciences
Department of Behavioural Medicine
The Faculty of Medicine, University of Oslo

In collaboration with
Department of Pharmacology, Oslo University Hospital
and
Department of Cardiology, Vestfold Hospital Trust

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Popular science summary in Norwegian

Hjerteinfarkt rammer omlag 12 000 mennesker i Norge hvert år og har betydelige negative konsekvenser for enkeltindividet, deres nærmeste, helsevesenet og samfunnet. Forhøyede kolesterolverdier har blitt identifisert som en av de mest sentrale årsakene til hjerteinfarkt og annen åreforkalkningssykdom i hjerte- og kar systemet. Det finnes i dag effektive legemidler som reduserer høyt kolesterol og bedrer prognosen til disse pasientene. Statiner utgjør den viktigste gruppen slike medikamenter. Store studier har gjentatte ganger vist at statiner effektivt reduserer risikoen for hjerteinfarkt og død både hos pasienter med etablert hjerte- og kar sykdom (sekundærforebyggende behandling) og blant de med høy risiko for slik sykdom (primærforebyggende behandling). Kombinasjonen av god effekt og lav pris gjør at statinene er svært kostnadseffektiv behandling. Statiner er derfor i dag en hjørnestensbehandling som anbefales sterkt til alle pasienter med etablert åreforkalkningssykdom. Det mest brukte statinet, atorvastatin, brukes i dag av ca. 380 000 mennesker i Norge.

Selv om statiner er svært effektive, er det mange pasienter som ikke tar medisinen som foreskrevet eller som slutter helt å ta de. Dette kalles redusert etterlevelse. Forekomsten av redusert etterlevelse med statinbehandling varierer betydelig mellom ulike studier (10-50%). Denne variasjonen skyldes sannsynligvis reelle forskjeller mellom ulike pasientgrupper og måletidspunkter, men det kan også skyldes at etterlevelse er definert forskjellig i ulike studier. Det er en utfordring i dag at vi mangler en felles definisjon av redusert etterlevelse og gode, objektive målemetoder som kan benyttes i klinisk praksis.

En sentral årsak til redusert etterlevelse med statiner er at pasientene opplever bivirkninger. Selv om alvorlige bivirkninger er svært sjeldne, rapporterer 10-25% av pasientene muskelsymptomer (smerter, kramper, stivhet, stølhet, svakhet) når de bruker statiner. Dette kalles ofte statin-assosierte muskelsymptomer (SAMS). I store kliniske legemiddelstudier, der deltagerne trakk lodd til å enten få statin eller identisk placebotablett uten statin (blindet behandling), har man imidlertid ikke sett

forskjell i forekomsten av SAMS mellom placebo og statin. Denne forskjellen mellom klinisk praksis og funn i legemiddelstudier gjør at det har hersket usikkerhet rundt hvorvidt muskelsymptomene faktisk skyldes statinet eller om plagene har en annen årsak.

I dette PhD prosjektet ønsket vi å utvikle en objektiv metode for å kunne måle etterlevelse til behandling med atorvastatin basert på konsentrasjonen av legemiddel i blodet (direkte måling). Videre undersøkte vi sammenhengen mellom etterlevelse målt med den nye direkte metoden, kolesterolverdier og selvrapporteringsverktøy for etterlevelse. Til slutt undersøkte vi effekten av atorvastatin på muskelsymptomer hos pasienter som tidligere hadde opplevd slike plager under behandling med atorvastatin for å avklare hvorvidt plagene skyldtes medisinen eller ikke. Alle pasientene som deltok i studiene hadde etablert åreforkalkning i hjertets kransårer, såkalt koronarsykdom.

I første delarbeid undersøkte vi 25 pasienter som brukte ulike doser atorvastatin. 12 av pasientene ble instruert til å slutte med medisinen (redusert etterlevelse) og vi målte deretter konsentrasjonen av atorvastatin og dets nedbrytningsprodukter i blodet de påfølgende tre dagene. Blodprøvene ble sammenlignet med de øvrige pasientene som tok atorvastatin som foreskrevet (normal etterlevelse). Vi viste at summen av atorvastatin og nedbrytningsprodukter kunne skille redusert etterlevelse fra normal etterlevelse med høy grad av presisjon.

I andre delarbeid benyttet vi metoden fra første delarbeid til å undersøke sammenhengen mellom direkte målt etterlevelse, tre ulike selvrapporterte mål for etterlevelse og kolesterolverdier hos 373 koronarpasienter som hadde deltatt i observasjonsstudiet NOR-COR. Alle pasientene hadde fått foreskrevet atorvastatin. På gruppenivå var det godt samsvar mellom grad av etterlevelse målt med den direkte metoden og kolesterolverdier. Det var imidlertid kun 22% til 40% av pasientene som hadde redusert etterlevelse målt direkte som også rapporterte lav etterlevelse på spørreskjemaet.

I tredje delarbeid kartla vi 984 hjerteinfarktpasienter og fant at nesten ti prosent rapporterte om muskelbivirkninger som de mente var forårsaket av behandling med atorvastatin. Til sammen 77

pasienter med selv-opplevde muskelbivirkninger deltok deretter i et klinisk forsøk der de fikk 7 ukers behandling med atorvastatin 40 mg/dag og 7 ukers behandling med en identisk placebotablett. Rekkefølgen ble bestemt med loddtrekning og hverken deltagerne eller vi som administrerte studien visste hvilken behandling deltagerne fikk. Dette kalles en randomisert, dobbelblindet overkrysningsstudie. Pasientene besvarte et spørreskjema ved oppstart og rapporterte muskelplager ukentlig i en dagbok i hele studieperioden. Vi målte også nivåer av atorvastatin i blodprøver for å undersøke om dette kunne egne seg som test for å identifisere pasienter med reelle muskelbivirkninger. På gruppenivå fant vi ingen forskjell i intensiteten av muskelsymptomer mellom periodene der de fikk behandling med atorvastatin og placebo. Noen pasienter opplevde mer plager under behandling med atorvastatin enn placebo, noen pasienter opplevde mer plager når de fikk behandling med placebo, mens de fleste opplevde like mye plager uavhengig av hvilken behandling de fikk. Det var ingen sammenheng mellom blodnivåer av atorvastatin eller dets nedbrytningsprodukter og muskelsymptomer ved behandling med atorvastatin.

Konklusjoner: Vi har utviklet en ny metode som ser ut til være godt egnet til å måle etterlevelse til behandling med atorvastatin direkte i blodet. Metoden fanger sannsynligvis opp flere pasienter med redusert etterlevelse med statinbehandlingen enn spørreskjemaer. Hos pasienter som rapporterer muskelplager ved behandling med atorvastatin var det ingen sammenheng mellom atorvastatin og muskelsymptomer når dette ble testet i en randomisert og blindet studie. Avhandlingen har bidratt med nye metoder og ny kunnskap om etterlevelse og bivirkninger ved behandling med atorvastatin som på sikt kan bidra til å skreddersy og forbedre behandling og oppfølging av koronarpasienter med potensielle effekter på helse og livskvalitet.

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In memory of Knut Kristiansen.

Articles in the thesis

This PhD thesis is based on the three publications listed below. The studies were conducted at the Department of Medicine at Drammen Hospital, Vestre Viken Hospital Trust, Department of Cardiology, Vestfold Hospital Trust, and Department of Pharmacology at Oslo University Hospital in close collaboration with the Department of Behavioural Sciences in Medicine at the University of Oslo.

Paper 1

A novel direct method to determine adherence to atorvastatin in patients with coronary heart disease.

Kristiansen O, Vethe NT, Fagerland MW, Bergan S, Munkhaugen J, Husebye E. Br J Clin Pharmacol. 2019;85(12):2878-2885.

Paper 2

The relationship between directly measured statin adherence, self-reported adherence measures and cholesterol levels in patients with coronary heart disease.

Kristiansen O, Sverre E, Peersen K, Fagerland MW, Gjertsen E, Gullestad L et al. Atherosclerosis. 2021;336:23-29.

Paper 3

Effect of atorvastatin on muscle symptoms in coronary heart disease patients with self-perceived statin muscle side-effects: a randomized, double blinded crossover trial.

Kristiansen O, Vethe NT, Peersen K, Fagerland MW, Sverre E, Prunés Jensen E et al. Eur Heart J Cardiovasc Pharmacother. 2021;7(6):507-16.

Abbreviations and definitions

ALT: Alanine aminotransferase

CHD: Coronary heart disease

CI: Confidence interval

CVD: Cardiovascular disease

CK: Creatinine kinase

DOT: Directly observed therapy

HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A

IQR: Interquartile range

LC-MS/MS: Liquid chromatography tandem mass spectrometry

LDH: Lactate dehydrogenase

LDL-C: Low-density lipoprotein cholesterol

MMAS-8: 8-item Morisky medication adherence scale

MUSE: MUscle Side-Effects of atorvastatin in coronary patients trial

NOR-COR: Norwegian coronary prevention study

PDC: Proportion of Days Covered

RCT: Randomized controlled trial

SAMS: Statin-associated Muscle Symptoms – subjective muscle complaints with normal or minor increases of blood CK concentrations succeeding the initiation of statin treatment or an increase if dose

SD: Standard deviation

VAS: Visual-analogue scale

Thesis summary

Background

Statins represent a cornerstone in the pharmacological prevention of coronary heart disease (CHD). Although highly efficacious and safe, poor adherence to treatment is common, contributing significantly to morbidity and mortality. There is no general agreement on the definition of poor adherence and valid methods to measure adherence are lacking. This reduces our ability to identify patients with poor adherence and thus implement evidence-based interventions shown to improve adherence. Statin-associated muscle symptoms (SAMS) are frequently reported by statin treated patients and constitute an important reason for poor adherence. However, it is uncertain to what extent these symptoms are caused by the statin as such complaints are reported at similar rates during treatment with statin and placebo in the hallmark statin randomized controlled trials (RCTs). The present PhD project therefore aimed to: i) develop an objective drug exposure variable (direct method) that allows discrimination among adherence, partial adherence and non-adherence to atorvastatin, ii) explore the relationship between the direct method, self-reported measures of adherence and blood lipid levels and, iii) to estimate the effect of atorvastatin on muscle symptom intensity in patients with subjective SAMS and determine the relationship with blood levels of atorvastatin and/or metabolites.

Methods

The studies comprising this thesis were conducted from 2011 to 2019 at Drammen and Vestfold hospitals, Norway. All study participants had established CHD and were using atorvastatin for secondary prevention. Atorvastatin and its major metabolites were measured at Oslo University hospital using a liquid chromatography and tandem mass spectrometry (LC-MS/MS) assay specifically designed for measuring adherence developed by our research group.

In paper one, 25 outpatients were recruited to a proof-of-concept clinical adherence study. Baseline adherence was ensured in all participants, and 12 (test group) were instructed to stop taking

atorvastatin to simulate non-adherence and return for blood sampling at the same time-point for three subsequent days. The remaining participants constitute the adherent control group. We explored individual metabolites and metabolites sums as measures of systemic atorvastatin exposure. Then, we compared blood concentrations between the test and control group at different time points to attempt to obtain cut-off values that allows discrimination between adherence (0-1 doses omitted), partial adherence (≥2 doses omitted) and non-adherence (>3 doses omitted).

In paper two, we performed a post-hoc analysis of the cross-sectional Norwegian Coronary Prevention (NOR-COR) study. In all, 373 patients answered a comprehensive self-report questionnaire containing three measures of adherence (Statin adherence past seven days, 8-item Morisky medication adherence scale (MMAS-8) and the Gehi et al. adherence question), underwent a clinical examination and had blood samples obtained median 16 month after a CHD event. At the time of data collection, none of the participants were aware that the blood samples later would be analyzed for adherence using the direct method.

In paper three, we identified 982 consecutive patients with previous or ongoing atorvastatin treatment. Of these, 97 (9.9%) reported SAMS and 77 were randomized to a double-blinded sequence of atorvastatin 40 mg/day and placebo lasting for 7 weeks each. Each treatment sequence was interrupted by a 1-week washout period. All participants had previous or ongoing muscle symptoms attributed to atorvastatin treatment. Muscle symptoms were recorded weakly in a diary on a 0 (no symptoms) to 10 (worst imaginable symptoms) visual-analogue scale (VAS). The primary outcome was the individual mean difference in muscle symptom intensity between the treatment periods. We also correlated blood concentrations of atorvastatin and/or metabolites to differences in muscular symptom intensity among patients with confirmed SAMS, defined as at least 25% and ≥1 cm higher symptom intensity during atorvastatin than placebo, to evaluate these blood concentrations as possible markers of statin-dependent muscle symptoms.

Results

In paper one, the sum of atorvastatin and all five metabolites were highly correlated to the ingested atorvastatin dose (Spearman's rho 0.71, 95% CI 0.44 to 0.87). A dose-normalized cut-off value of 0.10 nmol/L discriminated partial adherence from adherence with 92% sensitivity and 100% specificity. The metabolite 2-OH atorvastatin acid at a concentration of 0.014 mmol/L provided a suitable cut-off for non-adherence.

In paper two, 8% of the participants had reduced adherence (partial or non-adherence) as determined by the direct method developed in paper 1. Of these, 40%, 32% and 22% self-reported correspondingly on the statin adherence question, MMAS-8 and the Gehi et al. adherence question, respectively. The overall agreement between the direct method and the self-report measures of adherence was fair to moderate (Cohen's kappa 0.2 for the MMAS-8 to 0.4 for the statin last week question). Participants classified with reduced adherence had higher low-density lipoprotein cholesterol (LDL-C) concentrations in blood than those classified as adherent (2.8 vs. 1.9 mmol/L, $p<0.001$).

In paper three, atorvastatin had no effect on the intensity of muscle symptoms (mean VAS difference (statin-placebo) 0.31, 95% CI -0.24 to 0.86). Twenty (28%) participants experienced more symptoms during atorvastatin than placebo, 39 (55%) had the same symptom intensity, and 12 (17%) experienced more symptoms during placebo than statin. There were no differences in pharmacogenetic, demographic or clinical factors between those who fulfilled our predefined definition of confirmed SAMS, and those who did not. In the participants with confirmed SAMS, we found no relationship between muscle symptom intensity and blood concentrations of atorvastatin and/or metabolites.

Conclusions

This thesis introduces a novel direct method with cut-off values allowing discrimination among adherence, partial adherence and non-adherence to atorvastatin therapy. The direct method reflects

blood lipid levels and the agreement with self-reported adherence measures was fair to moderate. Thus, the direct method reveals more patients with reduced adherence than self-report measures. In patients with previous or ongoing muscle complaints attributed to atorvastatin therapy, there were no effect of high-intensity atorvastatin on muscle symptom intensity upon blinded re-challenge. Blood concentrations of atorvastatin and/or metabolites were not useful as discriminators of statin-dependent muscle symptoms. Overall, the thesis provides novel measurement methods and extends our understanding about adherence and side effects of atorvastatin in patients with CHD. This knowledge may contribute to individualize and improve current clinical practice for treatment and follow-up care for these patients with potential beneficial long-term effects.

1. Introduction

1.1 History, biological and clinical effects of statins

In 1976 Japanese biochemist Akira Endo (b. 1933) discovered ML 236B (compactin), a substance inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the human cholesterol biosynthesis (1). Compactin development progressed during the late 70s, and studies showed significant cholesterol lowering effect in dogs and monkeys (2, 3). In 1980 Kendo, together with physician Akira Yamamoto, reported that compactin lowered blood cholesterol levels in a patient with familial hypercholesterolemia (4). Their work on compactin did never materialize into a commercially available drug due to allegations of increased incidence of lymphoma in dogs treated with very high doses (5). However, the cholesterol lowering properties of compactin sparked interest from several pharmaceutical companies, and in February of 1979 the pharmaceutical company Merck and Akira Endo both isolated compounds similar to compactin (6). The compounds turned out to be the same substance, later named lovastatin. After clinical testing of cholesterol lowering effects and safety, the first commercially available statin was born when the FDA approved lovastatin for clinical use in 1987. Early fungal metabolite-derived statins were then followed by the advent of synthetic statins, and in 2001 the FDA approved atorvastatin (7). Today, atorvastatin is one of the most commonly used prescription drugs worldwide.

By competitive inhibition of HMG-CoA reductase, statins reduce hepatic cholesterol biosynthesis and increase the number of hepatic LDL receptors. In, turn this leads to an increase in the hepatic uptake of LDL-C and substantial reductions in LDL-C blood concentrations (8). Atorvastatin ≥40 mg/day typically reduce blood concentrations of LDL-C by approximately 50% and increase HDL-C by up to 10% (8-10). In addition to LDL-C lowering effects, statins also exert other potentially beneficial effects on the cardiovascular system, so called pleiotropic effects. These include improved endothelial function (11), anti-inflammatory effects (12, 13), and anti-thrombotic effects (14, 15).

LDL-C reductions by statins have consistently been shown to result in lower rates of cardiovascular events and mortality (16-18) and meta-analyses of randomized trials have found that a 1 mmol/L reduction in LDL-C results in a 20% average relative risk reduction for major vascular events (10). In a setting of secondary prevention, treating 10 patients for 5 years would typically prevent one patient from having a vascular event (10). Together with data from observational and genetic studies, statin RCTs have paved the way for establishing a causal relationship between LDL-C and atherosclerotic CVD (19).

1.2 Statins in clinical practice

Statins form the bedrock of modern pharmacological cardiovascular disease (CVD) prevention, and in patients with established CHD they have the highest level (1A) of recommendation (20). As statins are recommended to an increasing number of patients at risk of developing atherosclerotic disease and LDL-C treatment targets are continuously lowered, the number of statin users is rapidly increasing. Today, approximately 10% of the Norwegian population is prescribed a statin and atorvastatin is by far the most common with >380 000 users in 2020 (21).

Despite the strong evidence supporting their use, 7-12% of Norwegian CHD patients from routine clinical practice do not use statin therapy at all and only 43% reached the previously recommended LDL-C treatment target of <1.8 mmol/L (22, 23). The large European survey EUROASPIRE IV found an even poorer goal attainment with 20% of CHD patients on statins reaching target LDL-C (24). Possible causes of poor LDL-C control may relate to the physician (prescribing to low doses, reduce doses or forget to intensify treatment when appropriate), the patient (pharmacokinetic or pharmacodynamics variations reducing statin effect or non-adherent behavior) or the treatment itself (side effects) (22). Poor self-reported adherence and statin side effects have been identified as the major factors associated with not reaching the recommended LDL-C treatment target in Norwegian CHD patients (22). Furthermore, Norwegian primary care physicians report

insufficient knowledge on how to deal with statin side effects as an important barrier to optimal secondary prevention of atherosclerotic CVD (25).

1.3 Statin adherence

Adherence has been defined by the WHO as "the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" (26).

In Europe, poor adherence to cardiovascular medications is estimated to cause 9% of all CVD events (27). Poor statin adherence is associated with an increased risk of adverse cardiovascular events, and for patients with CVD, a graded inverse association between adherence and mortality has been shown (28, 29). Hence, poor medication adherence is a public health concern increasing health care costs by billions of dollars annually (30, 31).

Prevalence estimates for statin adherence vary significantly across populations and methods used for measuring adherence and the definition applied (32, 33). Poor adherence is undoubtedly common and has been described as a pandemic (34). In patients using statins for the secondary prevention of CHD, international registry data shows adherence rates of 50-80% approximately one year after a CHD event (35-37). Even in well-conducted statin RCTs, treatment discontinuation occurred in 7-42% of participants (38, 39). In the cross-sectional NOR-COR study, 8% of Norwegian CHD patients reported not taking their statin every day and a Norwegian registry study found that 16% were no longer prescribed a statin 1 year after an acute CHD event (22, 40).

There are several methods for measuring adherence in studies and clinical practice, however, the lack of an agreed definition of poor adherence and a "gold standard" for measuring adherence make comparisons difficult (41). Methods are typically divided into direct and indirect methods (31). Table 1 provides a summary of common measurement methods and their major strengths and limitations. The direct methods are more objective, but often more costly an impractical in routine

clinical practice. The indirect methods are often simpler and easily applicable in clinical practice, however they tend to be more inaccurate and prone to bias (31, 42).

Table 1 – Overview of common methods for measuring adherence to medication (adapted from (31, 42))

Method	Strengths	Limitations	Parameter measured
Direct methods			
Measurement of drug and metabolites	• Accurate • Proves ingestion of drug • Fast and easy for patients and health care provider	• Price • Individual differences in drug metabolism • Drugs may be detected long after stopping due to long half-lives • "White coat adherence"[a] • Invasive	• Drug concentration
Direct observed therapy	• Most accurate	• Only possible for hospitalized or institutionalized patients • Patients may hide pills in their mouth	• Number of doses ingested
Indirect methods			
Pill counts	• Simple to perform	• Easy to manipulate by the	• Number of doses missed

	• Quantifiable	patient (pill dumping)	
Self-report questionnaires	• Simple • Easily available in the clinical setting • Inexpensive	• Results easy to manipulate by the patient • Recall bias	• Pre-defined cut-off value defining adherence dichotomously
Electronic databases and registries	• Objective • Data is easily obtained	• Prescription fill rates do not mean drug is ingested • Risk of misclassification; varying data quality	• Medication possession ratio • Proportion of days covered
Clinical response to treatment (i.e. biological effects)	• Simple and often performed as a part of routine follow-up	• Clinical response may be affected by other factors than the treatment in question	• Dependent on response assessed (e.g. LDL-C or blood pressure)
Electronic monitoring devices	• Precise • Results easy to quantify • Provides information on patterns of	• Expensive • Patients aware of monitoring • Does not verify actual drug intake	• Overall percentage of doses taken

	medication taking	

[a] Patients improving their adherence around the time of measuring

For statins, LDL-C has been recommended as a measure of adherence (43). This is appealing as LDL-C is often measured during follow-up of patients with CHD and thus readily available. However, there are considerable variations in LDL-C response between statin treated patients, also in patients with high self-reported adherence (44, 45). Furthermore, a baseline statin-naïve LDL-C concentration, which is required for a meaningful assessment of change in LDL-C concentration, is not always available for CHD patients.

The term adherence contain three key aspects of patients' behavior taking medications: initiation (accepting and starting treatment), execution (delayed or omitted intake), and persistence (intermittent or permanent stopping treatment). It is therefore difficult to obtain a single valid measurement method embracing all aspects of such a complex and dynamic phenomenon. In routine care, clinical judgement represents the most common way by which healthcare providers assess adherence to medication. Unfortunately, clinicians perform poorly at this task and tend to underestimate poor adherence (46).

Barriers preventing adherence to medication are often divided into factors related to *the patient* (socioeconomic status, education, age/gender, cultural/experiential beliefs, forgetfulness, cognitive decline, psychological factors), *the healthcare system* (communication, prescription practices, cultural/experiential beliefs, poor screening tools, time, care transitions, poor reimbursement systems) or *the treatment* itself (complexity, costs, side effects) (34). For statins, one of the most important reasons for poor adherence is muscle side effects perceived to be caused by the statin (47, 48). To obtain a better understanding of the complex problem of statin adherence and to develop

effective interventions improving adherence, we need objective measurement methods and further knowledge about muscle side effects (49).

1.4 Blood concentration of atorvastatin – a useful marker of adherence?

Atorvastatin is administered in its acid form and following extensive first-pass metabolism by the gut and liver, the oral bioavailability is approximately 15% (50). Doses range from 10 to 80 mg and the systemic half-life of atorvastatin is around 14 hours (8). Hepatic uptake is mediated primarily by an organic-anion-transporting polypeptide (OATP), a protein coded by the SLCO1B1-gene (51). The genetic variant rs4149056 (c.521T>C or *5) is associated with reduced SLCO1B1 activity and thereby higher blood levels of atorvastatin (52). In vivo, atorvastatin is converted into hydroxyl and lactone forms. Hydroxylation is catalyzed by CYP3A4 (Figure 1), an enzyme showing high inter-individual variation in activity (53). Lactone and acid forms undergo interconversions through intermediate acyl glucuronides (54). Clearance of atorvastatin is mainly hepatic (50).

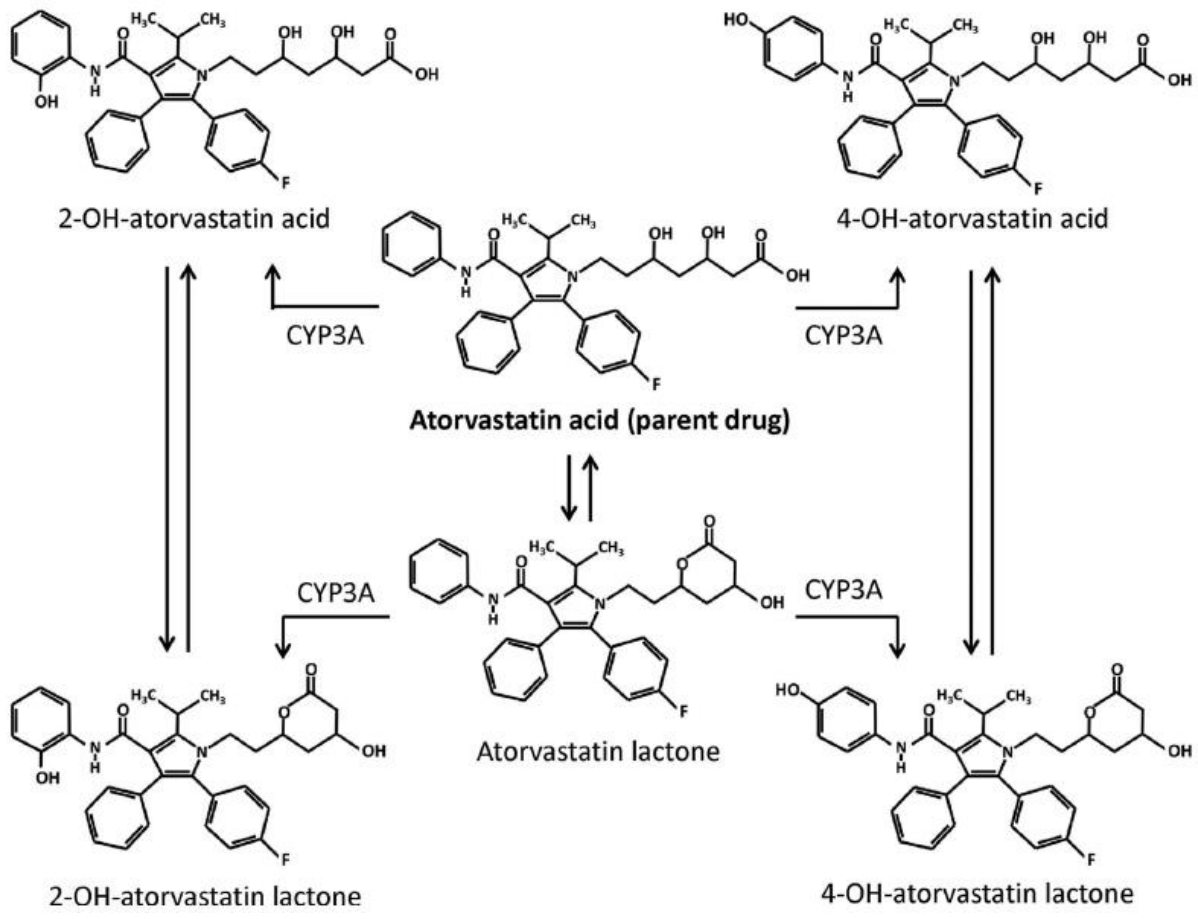

Figure 1 – Metabolism of atorvastatin in vivo

In light of lacking objective methods for measuring adherence to atorvastatin and its metabolism, our group developed an assay for quantifying the total exposure to atorvastatin and its five major metabolites using LC-MS/MS methodology (55). The method addresses issues of preanalytical stability (acid to lactone interconversions) by using the sums of acid and lactone forms and easy sample handling at ambient temperature makes it feasible for measuring adherence (55). However, an algorithm with cut-off values corresponding to clinically meaningful adherence is required prior to use in future research and clinical practice.

1.5 Side effects of statins

Several large-scale randomized trials have shown that statins have a low risk of serious side effects (10). However, increases in HbA1c, slight excess risk of cerebral hemorrhage and rare muscle side effects with large increases in blood CK concentrations are adverse statin evidenced by RCTs (9, 17, 56). Furthermore, an excess increase in liver transaminases occurs with certain statins, including atorvastatin (57).

Myopathy, characterized by proximal muscle symptoms and four to 10-fold increases in blood CK concentrations from the upper normal limit, occur in approximately 1 per 10 000 statin-treated individuals per year (58). Rhabdomyolysis, a more severe form of myopathy with increases in CK by more than 40 times the upper normal limit, occurs even more rarely with an estimated incidence of 2-3/100 000 patients treated per year (58). The risk of myopathy is increased with high-dose regimens (ie. simvastatin 80 mg, which is no longer recommended) and typically resolve upon stopping treatment (59).

Proposed mechanisms by which statins may cause muscle symptoms include changes in the stability and fluidity of muscle cell membranes, protein signaling, impaired mitochondrial function and reduced cell-membrane cholesterol content (60). Previous studies have also suggested a

relationship between elevated levels of statin metabolites, in particular lactone metabolites, and muscle side effects of statins (61-63).

Other muscle symptoms, collectively called SAMS, include pain, aching, weakness, stiffness, cramps and tenderness typically occurring upon statin initiation or an increase in dose (47). SAMS differs from myopathy and rhabdomyolysis in that symptoms are accompanied by normal or only slightly increased blood CK concentrations (<4-10 times the upper normal limit). In open non-randomized studies, SAMS are reported by 10-25% of statin users and occur more often in statin treated individuals than those who are not (64-67). In blinded RCTs, however, SAMS occur with similar frequency in statin and placebo treated individuals (57). On one hand, inherent biases of observational studies hamper their ability to establish causal relationships. On the other hand, statin RCTs may have limitations in detecting side effects due to run-in periods, strict selection of participants and poor measures of muscle symptoms. Furthermore, patients expecting side effects may be more likely to experience them, a phenomenon known as the nocebo effect. For statins, this effect is well established, and likely contributes to the discrepancy in SAMS incidence between RCT and observational studies (68). Along these lines, a Danish study found that negative coverage of statins in the media was associated with treatment discontinuation and an increase in CVD events whereas the opposite was observed with positive media coverage (69).

To confirm whether the statin causes subjective muscle symptoms, blinded crossover trials exposing participants to statin and placebo in random order, may be useful. A small randomized trial, (n=8) exposing participants with SAMS to several periods of statin or placebo, found no relationship between muscle symptom intensity and the statin (70). Two larger crossover RCTs designed to test the effect of non-statin therapies in selected SAMS patients, confirmed a relationship between the drug and muscle symptoms in 36 to 43% of participants. Thus, to what extent, if any, statins cause muscle symptoms without objective signs of myonecrosis, remains uncertain. Although algorithms to

diagnose and manage patients with SAMS have been proposed, the lack of a clinically useful biomarker makes the clinical management of these patients challenging (47, 71, 72).

1.6 Summary of knowledge gaps and rationale for the thesis

To quantify and understand the challenge of poor adherence to statins, valid measurement methods are required. Our group has recently developed a direct method allowing precise measurement of atorvastatin and five metabolites in blood. It is unknown whether the direct method is feasible and valid for assessing adherence in clinical practice and cut-off values reflecting clinically relevant adherence are therefore required. Moreover, the relationship between the direct method and indirect measures of statin adherence (i.e. LDL-C and self-report measures) remains to be determined. Novel measurement methods and improved understanding of the adherence phenomenon may provide insight into potentially modifiable factors associated with non-adherent behavior, and pave way for novel interventions to improve statin adherence. Previous studies have identified muscle side effects as an important reason for poor statin adherence. The discrepancies between observational and RCT data regarding statin use and SAMS, remain a concern, and the extent to which high-intensity statins cause muscle symptoms in patients with SAMS is unknown. Finally, if the SAMS phenomenon is caused by the statin, diagnostic biomarkers are yet to be identified.

2. Aims and hypothesis of the thesis

The overall aim of this thesis is to provide new insight about adherence to atorvastatin treatment, muscle side effects and their relation to blood concentrations of atorvastatin and metabolites. In turn, this may translate into clinically useful tools to personalize and improve statin management.

2.1 Aims and hypothesis for paper 1

To develop a drug exposure variable with cut-off values that may discriminate adherence from reduced adherence (partial and non-adherence) to atorvastatin treatment in patients with CHD. Based on the pharmacokinetics of atorvastatin in blood, we hypothesized that adherence could be discriminated from reduced adherence after omitting two or more doses.

2.2 Aims and hypothesis for paper 2

To evaluate the relationship between directly measured atorvastatin adherence, self-reported adherence measures, and blood cholesterol concentrations in patients with CHD. To compare the proportion with reduced statin adherence measured by indirect and direct methods, and to identify clinical and psychosocial factors associated with non-adherence as determined by the direct method. We hypothesized that the direct method would identify more patients with reduced adherence than self-report questionnaires and correlate better to LDL-C concentrations. Furthermore, we hypothesized that potentially modifiable determinants of reduced adherence could be identified by using the direct method.

2.3 Aims and hypothesis for paper 3

First, to determine the effect of atorvastatin on muscle symptom intensity in CHD patients with subjective muscle symptoms self-attributed to atorvastatin. Second, to determine the relationship between blood concentrations of atorvastatin and muscle symptom intensity, and to evaluate the diagnostic properties of blood concentration of atorvastatin for classification of truly statin-dependent muscle symptoms. We hypothesized that 30-40% of the patients would report

significantly higher muscle symptom intensity during treatment with atorvastatin compared to placebo. We also hypothesized that this subgroup of the SAMS population would have higher levels of atorvastatin and/or metabolites that their counterparts without placebo-controlled SAMS.

3. Materials and methods

This thesis is based on three different studies: A proof-of-concept pharmacokinetic adherence study, a cross-sectional study and a randomized crossover trial. There are important differences in the methods used between the three studies. Therefore, I have decided to describe overlapping methods between studies first before describing study-specific methods.

3.1 Population and setting

We recruited study participants from two secondary care Norwegian hospitals, Drammen and Vestfold. The total catchment area of these hospitals has approximately 410 000 inhabitants comprising 7.6% of the Norwegian population. The catchment area constitute both rural and city areas and is representative of Norwegian economy, age distribution, morbidity and mortality (73). All participants had established CHD and the vast majority had experienced a myocardial infarction. Flow-chart and key baseline characteristics for all participants is provided in Figure 2 and Table 2 below.

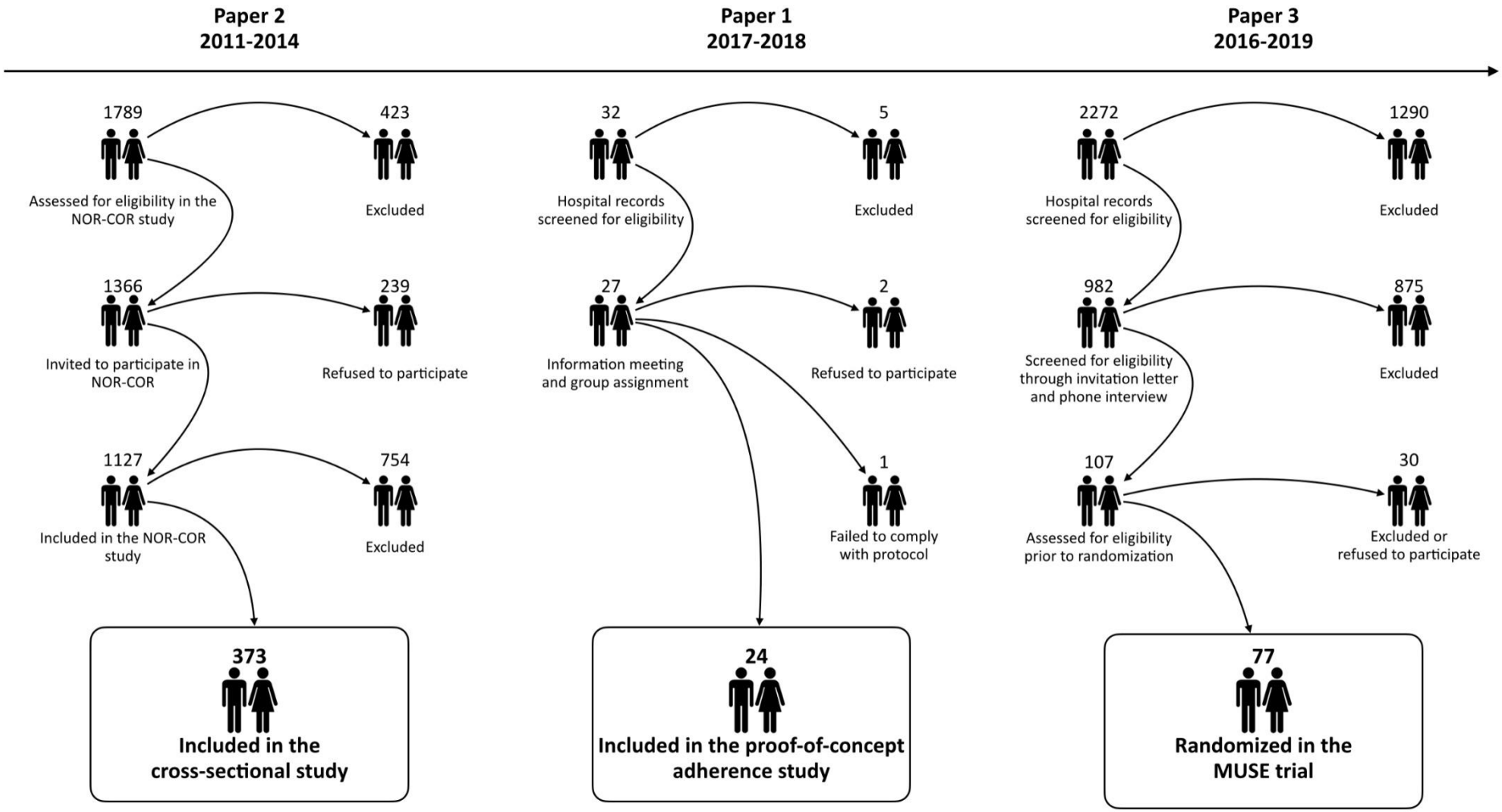

Figure 2 – flow chart for all study participants

Table 2 – Baseline characteristics of all study participants

Characteristic	*Paper 1 (n=24)*	*Paper 2 (n=373)*	*Paper 3 (n=77)*
Age, mean (SD)	66.1 (10.6)	63.0 (9.1)	63.7 (9.5)
Female gender, n (%)	5 (20.8)	70 (18.8)	26 (33.8)
Low education[a], n (%)	-	245 (65.7)	49 (63.6)
Myocardial infarction as index event, n (%)	20 (83.3)	321 (86.1)	64 (83.1)
LDL-C in mmol/L, mean (SD)	2.2 (0.6)	1.9 (0.7)	2.5 (1.1)
Prescribed statin at baseline, n (%)	24 (100)	373 (100)	68 (88.3)
Self-perceived SAMS, n (%)	4 (16.7)	25 (6.7)	77 (100)

[a] Defined as completion of primary and secondary school only.

3.2 Quantification of atorvastatin in blood

To quantify atorvastatin we applied liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). LC-MS/MS is currently the gold standard method for bioanalysis of pharmaceuticals, as it allows fast, highly selective and accurate quantification of drugs and metabolites. It is therefore routinely used in clinical practice for therapeutic drug monitoring worldwide. A brief description of LC-MS/MS methodology is provided below.

Liquid chromatography separates compounds in a flowing liquid (mobile phase) based on their physical and chemical interaction with an immobile material (stationary phase). The fundamental purpose of liquid chromatography is compound separation. However, liquid chromatography also allows to some extent identification of different chemicals through analysis of the time used by the chemical to pass through the stationary phase, known as retention time. The retention time may be manipulated through selection of different compositions of the mobile phase and different types of stationary phases. After compound separation (i.e. clean-up for practical

purposes) by liquid chromatography, the compounds are vaporized and charged by electrospray ionization. Sophisticated mass spectrometry quadrupoles then select ions of interest based on their mass to charge ratio. The selected ions are fragmented in a collision cell and the daughter ions pass through to the detector and are quantified. The process consisting of liquid chromatography and tandem mass spectrometry with fragmentation thus results in very high analytical selectivity.

In a method development preface, our group established an assay allowing fast and reliable quantification of the acid and lactone form of atorvastatin (parent drug), ortho- (2-OH) and para- (4-OH) hydroxyl atorvastatin (55). The assay is accurate (92-110%) and precise (≤8.1%) over a wide range of concentrations and validated according to the EMA Guideline on bioanalytical method validation (74). To explore the relationship with muscular side effects in paper 3, we also semiquantified atorvastatin acylglucuronide by calibration towards the instrument response of atorvastatin acid (not validated).

The interpretation of the LC-MS/MS analyses of atorvastatin were based on either quantification of individual metabolites or metabolite sums. Using metabolite sums has the advantage of counteracting preanalytical instability of lactone forms (55) and presumably variations in drug metabolism.

All LC-MS/MS analyses (Transcend II LX-2 TSQ Quantiva, Thermo Fisher Scientific) in the studies constituting this thesis were performed at the Department of Pharmacology at Oslo University hospital.

3.3 Study variables

Table 3 – Overview of baseline study variables

Variable	Description	Paper
Acute myocardial infarction	Hospital medical records.	2,3
Age	Hospital medical records in all papers.	1,2,3

ALT	Analyzed on a clinical chemistry analyzed (Architect ci16200, Abbot Laboratories) at Drammen Hospital	1,3
Anxiety or depression (diagnosis)	Hospital medical records.	3
Arthrosis	Self-report questionnaire.	3
Atorvastatin dose	Self-report questionnaire and hospital medical records, crosschecked against electronic prescription module where applicable.	1,2
Albumin	Analyzed on a clinical chemistry analyzer (Architect ci16200, Abbot Laboratories) at Drammen Hospital	1
Any side-effects attributed to cardiovascular drugs	Self-report questionnaire.	2
Bergen Insomnia Scale	Self-report questionnaire. 6 items about sleep onset, maintenance of sleep and early morning wakening (75)	2
Body weight measured	Nearest 0.5 kg in light clothes without shoes (SECA 264, DE).	1,2
Body weight, self-reported	Self-report questionnaire.	3
Body mass index	Weight in kg/height in meters^2.	2,3
High sensitivity C-reactive protein	Analyzed on a clinical chemistry analyzer (Architect ci16200, Abbot Laboratories) at Drammen Hospital	2,3
Creatinine	As above.	3
Creatinine kinase	As above.	1,3
Charlston comorbidity index	Hospital medical records. Summarizes 16 somatic comorbidities and age category (76)	2
Current smoking	Self-report questionnaire.	2,3
*CYP3A4 (*22, rs35599367)*	Analyzed at Oslo University hospital for paper 1 and Drammen hospital for paper 3 (Light Cycler® 480, Roche Diagnostics)	1,3
*CYP3A5 (*3, rs776746)*	As above.	1,3

Diabetes mellitus	Hospital medical records. Includes type 1 and 2 diagnosis or treatment with antidiabetic medication.	2,3
Estimated glomerular filtration rate	Analyzed on a clinical chemistry analyzer (Architect ci16200, Abbot Laboratories) at Drammen hospital	3
Heart failure (HF)	Hospital medical records. Includes HF with reduced and preserved ejection fraction as defined in (77).	3
Hospital Anxiety and Depression Scale	Self-report questionnaire. 14 items on symptoms of anxiety (HADS-A) and depression (HADS-D) (78).	2
Hypo- or hyperthyroidism	Hospital medical records. Diagnosis or treatment with thyrostatic or hormone substitution.	3
Lactate dehydrogenase	Analyzed on a clinical chemistry analyzer (Architect ci16200, Abbot Laboratories) at Drammen Hospital.	1,3
Low-density lipoprotein cholesterol	Non-fasting venous blood samples collected in an EDTA tube. Analyzed on a clinical chemistry analyzer (Architect ci16200, Abbot Laboratories) at Drammen Hospital.	1,2,3
Low education	Self-report questionnaire. Defined as completion of primary and secondary school only.	2,3
Low-physical activity	Self-report questionnaire. Defined as physical activity less than 30 min of moderate intensity 2–3 times weekly.	3
Moderate- or low-intensity statin therapy	Self-report questionnaire crosschecked against hospital medical records and electronic prescription module. High-intensity statin therapy refers to regimens known to lower LDL-C on average 50% (79). All other statin regimens are considered low- or moderate intensity statin therapy.	3
Number of concomitant medications	Self-report questionnaires and hospital medical records.	1
NSAIDSs or analgesics	Self-report questionnaire.	3
Physical activity	As above.	3

Previous atorvastatin discontinuation due to side effects	As above.	3
*SLCO1B1 (*5, c521T>C)*	Analyzed at Oslo University hospital for paper 1 and Drammen hospital for paper 3 (Light Cycler® 480, Roche Diagnostics)	1,3
Time since last coronary event	Hospital medical records.	3
Type D negative affectivity score	Part of a DS-14 questionnaire with 14 items (80). 7 items on the negative affectivity subscale.	2
Type D social inhibition score	Part of a DS-14 questionnaire with 14 items (80). 7 items on the social inhibition subscale.	2
Stroke/transitory ischemic attack	Hospital medical records. Diagnosis.	3
Systolic blood pressure	Validated digital sphygmomanometer (Welch Allyn WA Connex ProBP 3400) (81). Measured by trained cardiac nurse.	2

3.4 Statistics

Descriptive statistics are presented as mean (SD) for normally distributed data, otherwise median (range, interquartile range) was used. Categorical data are presented as numbers (proportion in %). To compare mean differences across two groups, the student's t test with adjustment for unequal variance was applied to apparently normally distributed data. If markedly skewed data, transformations (log, ln) were attempted (assessed visually by Quantile-Quantile plots). If normality was not obtained, non-parametric methods of hypothesis testing were applied. Differences between categorical data were assessed by chi-square tests or the Fischer mid-p test. Diagnostic performances were assessed by Receiver Operating Characteristics (ROC) curves. P-values reported are two-sided and if less than 0.05 the corresponding difference is considered statistically significant. All statistical analyses were reviewed (paper 1 and 2), or conducted (paper 3) by a biostatistician. Analyses were performed using SPSS version 25/26, Stata/SE 16.0 and/or Matlab R 2014A.

3.5 Study-specific methods in paper 1

3.5.1 Population

Twenty-five CHD patients treated with atorvastatin 10 mg (N=5), 20 mg (N=6), 40 mg (N=7) and 80 mg (N=7) were recruited from the outpatient clinic at Drammen hospital in January 2018. Exclusion criteria were: i) CHD event within 2 years prior to inclusion, ii) symptoms of unstable CHD, iii) known difficulties with blood sampling, iv) likely not able to comply with the test procedure as assessed by the study physicians. We excluded one participant from the analyses due to non-adherence to the study protocol. Key baseline characteristics of the participants are shown in Table 2.

3.5.2 Design

The study was designed as a proof-of-concept clinical adherence study aimed to develop a variable representative of atorvastatin exposure and to provide cut-off values that could discriminate among adherence, partial adherence and non-adherence. Prior to study start, all patients participated in a meeting were thorough information regarding the background, aims and study protocol were provided prior to signing of an informed consent. To ensure steady state concentrations of atorvastatin prior to study start, we emphasized the importance of administering atorvastatin once daily between seven and 10 AM for at least 7 days prior to study start. All participants reported statin intake in a diary. At the first day of the study blood samples were collected one hour (t_{-1}) and immediately before (t_0) observed intake of atorvastatin (directly observed therapy – DOT) to exclude the possibility of an unscheduled morning dose (increase in drug concentration between t_{-1} and t_0). After the DOT, participants were consecutively assigned to the test or control group ensuring equal distribution of dosages in each group. The test group was then instructed to simulate reduced adherence by stopping intake of atorvastatin, and return for blood samples at exact same time point for three consecutive days. At day 4, the test group underwent a second DOT study to simulate escape dosing in non-adherent patients. Stability of lactone forms is improved by handling the blood samples at very low temperatures(55). However, this has disadvantages with regard to practical

handling in routine clinical practice. We therefore also handled a set of samples in ambient temperature to assess the cut-off values in samples handled according to this more practical procedure. Flow of participants and the test procedure is illustrated in Figure 3.

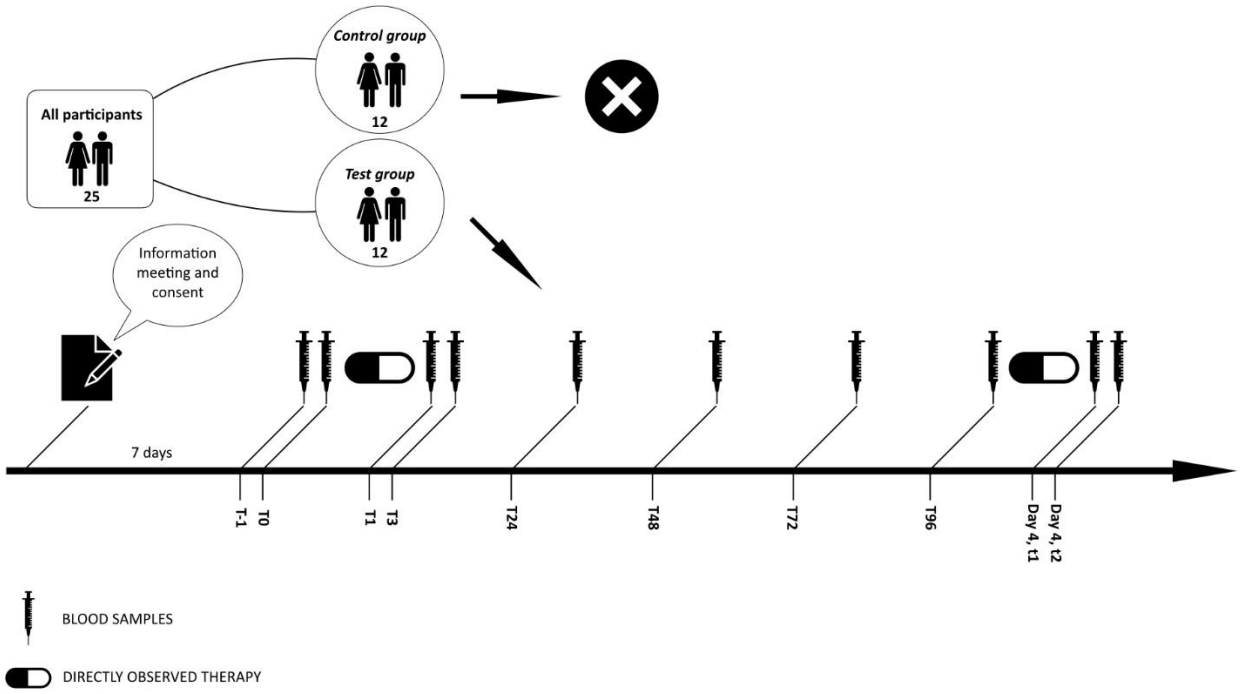

Figure 3 – Illustration of test procedure and participant flow in Paper 1

3.5.3 Defining partial and non-adherence

There is no consensus as to a definition of partial adherence and non-adherence in the statin literature. Hence, we agreed on selected presets to align cut-offs prior to data analyses based on the clinical and research experience of senior members of the NOR-COR group. First, to avoid adherent patients misclassified as partially adherent, we allowed omission of a single dose. Second, the cut-off between adherence and partial adherence should correspond to at least two days without drug intake. Third, the cut-off for non-adherence should correspond to more than three consecutive days without drug intake. These presets applied to the general pharmacokinetics of atorvastatin and its major metabolites may allow identification of degrees of adherence, which may prove to be clinically relevant.

3.5.4 Comparator

We compared blood concentrations of atorvastatin and five metabolites between the *test group,* simulating reduced adherence, and the adherent *control group* at the different time points. To account for preanalytical instability of lactone forms as well pharmacokinetic differences between patients, sums of metabolites were assessed as potential discriminators between adherence and partial adherence. Due to practical issues of study implementation, the study took place for five weekdays. Thus, empirical data on patients omitting doses for more than three days were not available. As a pragmatic approach to obtain a cut-off value for non-adherence corresponding to more than three days without drug intake, we extrapolated metabolite concentrations based on the observed half-life of individual metabolites. The instrument response corresponding to these estimated concentrations were assessed as potential discriminators between partial and non-adherence.

3.5.5 Statistics

As the participants were consecutively assigned to the test and control group on the basis of atorvastatin dose, hypothesis testing was therefore conducted to assess between-group differences in important baseline characteristics. Due to the small sample size, we used the Fischer mid-p test to assess differences in categorical data between the test and control group. Differences in blood concentrations of atorvastatin and metabolites were assessed by Mann-Whitney U test for unrelated samples, and for paired samples the Wilcoxon signed rank test was used. As a we did not expect a linear relationship between dose and systemic exposure to atorvastatin (82), we estimated this relationship by Spearman rank correlation coefficients. ROC-curve analyses were performed to identify cut-off values for partial adherence.

We calculated the elimination rate constant, and further on, the individual half-lives of atorvastatin and metabolites based on linear regression of the Ln-transformed blood concentrations vs. time.

3.6 Study specific methods in paper 2

3.6.1 Population

To address the aims of paper 2, we selected the Vestfold cohort from the NOR-COR study as only blood from these patients had been bio-banked. The NOR-COR study took place in 2014-2015 and included patients aged 18-80 years with a first or recurrent diagnosis or treatment of a CHD event (index event) (83). Patients were chronologically identified from hospital discharge lists from 2011-2014. This was done to allow time for adequate follow up after the index event (cardiac rehabilitation, titration of medication etc.), and to harmonize with previous and ongoing European surveys of cardiovascular risk factors (24). Finally, the choice of population allowed inclusion of participants within a relatively short time frame. Exclusion criteria in the NOR-COR study were: (i) a diagnosis of type 2 myocardial infarction, (ii) not able to understand the Norwegian language, (iii) cognitive impairment including living in nursing homes, (iv) psychosis, (v) drug abuse and (vi) short life expectancy (83). We included all participants prescribed atorvastatin at the index event and no information of change in statin treatment at follow up.

3.6.2 Design and study assessments

NOR-COR was a cross sectional study with a retrospective component. All participants answered a comprehensive self-report questionnaire, underwent a clinical examination and blood sampling at median 16 (range 2-36) months after the index event. The self-report questionnaire contained three measures of adherence: The MMAS-8, The Gehi et al. adherence question and a single question about statin adherence in the past seven days prior to participation. The MMAS-8 is a commonly used general measure of medication adherence. It consist of eight items that mainly capture non-adherent behavior. A score of less than six out of 8 points is considered to be consistent with reduced adherence (84). The Gehi et al. adherence question is a single question about medication adherence in the past month: “In the past month, how often did you take your medications as the doctor prescribed?” Possible responses were “All of the time” (100%), “Nearly all of the time” (90%),

"Most of the time" (75%), "About half the time" (50%), or "Less than half of the time" (<50%). For the present comparison with the direct method, participants responding "most of the time" or rarer, were classified with reduced adherence (85). Both the MMAS-8 and the Gehi et al. adherence question have previously been shown to predict clinical cardiovascular outcomes using the same cut-off values (84, 86). With the statin adherence question, we asked: "In the past seven days, how often did you take your statin as prescribed?" Possible responses were "Every day", "6/7 days", "5/7 days", "4/7 days" or "<4/7 days". Participants responding "5/7 days" or rarer were classified with reduced adherence as this corresponds to our definition of partial adherence in paper 1. In order to characterize the study population and to identify potentially modifiable factors associated with reduced adherence, we also explored several demographic, clinical and psychosocial variables as described in Table 3.

3.6.3 Comparator

We compared the resulting adherence classification between the self-report measures and the direct method developed in paper 1. The direct method allows discrimination among adherence, partial adherence (≥2 consecutive doses omitted) and non-adherence (>3 consecutive doses omitted). However, in the present study we merged participants classified with reduced adherence together with those classified as non-adherent. This was done for two reasons: First, there is no established and corresponding definition for partial adherence for the self-report measures. Second, the cut-off value for non-adherence by the direct method has important limitations due to its semiquantative nature. LDL-C concentrations in blood was compared among participants according to the classification of adherence by the different measurement methods. Furthermore, to validate our cut-offs developed in paper one, we also compared LDL-C among patients classified as adherent, partially adherent and non-adherent by the direct method.

3.6.4 Statistics

Differences in mean LDL-C concentrations among participants classified as adherent, partially adherent and non-adherent were tested using one-way ANOVA. Differences in clinical and psychosocial factors between participants classified with adherence vs. reduced adherence were explored with descriptive statistics and hypothesis testing as described under 3.4. Agreement between adherence classification by the direct method and the self-report measures were described with percentage agreement as well as by Cohen's kappa (≤0 - no agreement to 1 – perfect agreement). Calculation of kappa values was conducted using VassarStats: Website for statistical computing (87).

3.7 Study specific methods in paper 3

3.7.1 Population

We consecutively screened hospital discharge lists of patients with a diagnosis of a first or recurrent CHD event from Drammen and Vestfold hospitals between 2016 and 2019. In all, 2272 hospital medical records were examined and patients were excluded based on the study exclusion criteria as illustrated in Figure 2. The exclusion criteria included peripheral artery disease, any contraindication for atorvastatin, myopathy or liver failure with previous statin treatment (CK >10 x upper limit of normal range, ALT >3 x upper limit of normal range), short life-expectancy, unable to understand Norwegian, premenopausal females (childbearing potential) and/or participation in another randomized trial. Finally, we excluded patients with any condition or situation, which in the investigator's opinion could put the subject at significant risk, confound the study results, interfere significantly with the subject participation in the study, or render informed consent unfeasible.

After the initial screening of hospital medical records, we invited 982 potential participants to a telephone interview to assess potential eligibility. One hundred seven patients fulfilling eligibility criteria; self-reported muscle symptoms attributed to ongoing or previous treatment with atorvastatin, were invited to participate. In all, nine patients did not show up to baseline. At baseline,

all potential participants underwent an interview with two study physicians. The interview focused on the temporal relation between symptoms and atorvastatin exposure as well as a review of potential exclusion criteria prior to randomization. Eighteen patients were excluded at the baseline interview and three refused to participate allowing us to randomize 77 patients.

3.7.2 Design and intervention

3.7.2.1 The randomized trial – MUscle Side Effects of atorvastatin (MUSE)

The study was a randomized, double blinded, crossover study designed to test the effect of atorvastatin 40 mg on the intensity of muscle symptoms in CHD patients with previous subjective muscle symptoms attributed to atorvastatin. The MUSE study design, methods and pilot results are thoroughly elaborated in a separate publication (88). Participants were randomly assigned to seven weeks of atorvastatin in treatment period 1 and 7 weeks of placebo in period 2, or vice-versa (AB-BA crossover design). An overview of the design is provided in Figure 4 below. Participants experiencing intolerable muscle symptoms were allowed to discontinue their allocated treatment prior to seven weeks. However, we encouraged continuation for at least three weeks to ensure adequate data for assessment of the primary outcome.

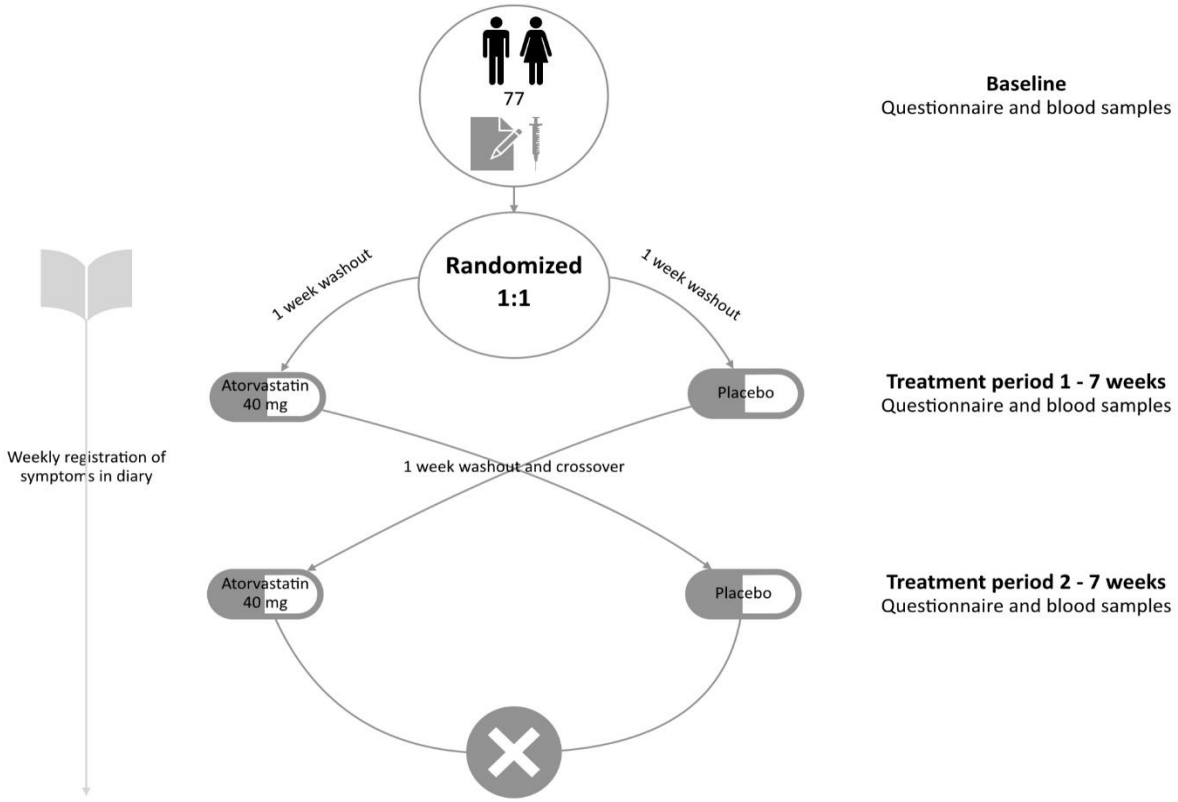

Figure 4 – Overview of study design and participant flow in Paper 3

3.7.2.2 The control group

The study also included an age and sex matched control group of CHD patients without any history of muscle complaints despite treatment with atorvastatin ≥40 mg. This group was assigned to seven weeks of open-label treatment with atorvastatin 40 mg to compare blood concentrations of atorvastatin and metabolites with the participants in randomized trial.

3.7.3 Data collection

Intensity of muscle symptoms (pain, tenderness, stiffness, cramps or weakness) was registered by the participants weekly in a diary on a VAS scale (0 – no symptoms to 10 – worst imaginable symptoms). On the last day of each treatment period, we obtained blood samples immediately prior to the next scheduled dose (C_0 – trough concentration) and two hours after observed tablet intake (C_2 – peak concentration). Participants were allowed a light meal prior to sampling of C_0 samples but fasted between observed tablet intake and blood sampling at C_2. We measured adherence to the

allocated treatment by counting remaining pills in returned containers (proportion of days covered (PDC) = (1 – number of remaining pills/number of pills delivered to patient) x 100%). We also measured adherence directly in blood by the direct method developed in paper 1 (classified as non-adherent at the end of the placebo period and adherent at the end of the atorvastatin period).

3.7.4 Randomization and blinding

We used an electronic randomization system to randomize participants 1:1 to double-blinded treatment with atorvastatin or matching placebo. Block sizes of four and six in random order, stratified according to study site and previous discontinuation of atorvastatin, were used to minimize the risk of imbalance between groups as the trial included a relatively low number of participants. The capsules, containing atorvastatin or placebo, were identical in appearance. We collected the containers at the end of the first treatment periods ot avoid participants attempting to compare capsules between the treatment periods. Results of all laboratory tests, including LDL-C, CK and ALT, were unavailable to all participants and the study personnel within the trial period.

3.7.5 Outcomes

The primary outcome was the individual difference in muscle symptom intensity between the treatment periods – defined as the difference between the VAS 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.

Secondary outcomes:

- Proportion with confirmed SAMS – 25% higher individual mean VAS-score during the atorvastatin period than the placebo period, and ≥1 cm absolute difference.
- Relationship between muscle symptom intensity and systemic exposure to atorvastatin - correlation between individual differences in mean muscle symptom intensity and concentrations of atorvastatin and metabolites in blood among patients with confirmed SAMS.

- Diagnostic properties of atorvastatin and metabolites for classification of truly statin dependent muscle symptoms – sensitivity, specificity and area under the ROC curve of blood concentrations of atorvastatin and metabolites for the classification of confirmed SAMS.
- Differences in mean blood concentrations of atorvastatin and metabolites in RCT participants defined as non-SAMS and the control group.
- Adherence to allocated treatment – pill counts (proportion of days covered) and direct measurement in blood at the end of each treatment period.

3.7.6 Statistics

We based our sample size calculation on the ability to detect a one cm difference in VAS score between the treatment period with atorvastatin and the period with placebo. In a previous study, a 1.3 cm change in VAS score, corresponded to 'a little more' or 'a little less' symptoms (lower limit of the 95% CI at 1 cm, SD 1.7 cm) (89). For 90% power to detect a difference of 1.0 (SD 2.5), using a one-sample T test, and for 80% power to detect a difference of 40% SAMS under statins vs. 15% SAMS under placebo, a sample size of *n*=68 was needed (McNemars test for paired probabilities). We planned to include 80 participants, thereby accounting for potential dropouts and protocol deviations. All statistical analyses were pre-specified, except where noted in the paper, and described in detail in the Statistical Analysis Plan (90). The primary outcome (95% CI) was estimated by linear regression with change in symptom intensity between treatment periods as the dependent variable and previous atorvastatin discontinuation and study site as covariates. As there is only one primary analysis in the trial, all other analyses are considered supportive or exploratory. The relationship between muscle symptom intensity and blood concentrations of atorvastatin and metabolites was estimated using Spearman rank correlation coefficients.

4. Results

4.1 Paper 1

Baseline clinical and demographic characteristics were not different between the test and control groups (p-values ranged from 0.15 to 0.94). The sum of atorvastatin and all metabolites correlated to a high degree with the atorvastatin dose administered as shown in Table 4.

Table 4 – Correlation between blood plasma concentration of atorvastatin and/or metabolites and atorvastatin dose in Paper 1

Analyte	**Spearman's rho**	**95% CI**
ATV acid (parent drug)	0.598	0.257 – 0.806
ATV acid and lactone	0.587	0.241 – 0.800
2OH ATV acid and lactone	0.752	0.501 – 0.886
4OH ATV acid and lactone	0.713	0.435 – 0.867
ATV acid + 2OH ATV acid and lactone	0.697	0.409 – 0-858
ATV acid + 4OH ATV acid and lactone	0.626	0.298 – 0.821
ATV acid + all five metabolites	0.714	0.437 – 0.867
Obtained using Spearman's correlation using t_0 samples handled at low temperature. ATV, atorvastatin; 2OH, ortho-hydroxyl, 4OH, para-hydroxyl.		

Calculated half-lives were median 14 (range 11-24) hours for atorvastatin acid, 13 (10-20) hours for atorvastatin lactone, 15 (12-48) hours for 2-OH atorvastatin acid, 15 (11-37) hours for 2-OH atorvastatin lactone, 21 (14-42) hours for 4-OH atorvastatin acid, and 19 (13-40) hours for 4-OH atorvastatin lactone.

The dose-normalized atorvastatin plus metabolites concentrations separated all subjects in the partially adherent *test group* from the adherent controls at 0.18 (nmol/L)/mg after 3 days without atorvastatin for the samples handled in ambient temperature (Figure 5). To minimize the risk

of classifying adherent patients as partially adherent, we suggest a cut-off value of 0.10 (nmol/L)/mg (Figure 5). 2-OH atorvastatin acid with instrument response corresponding to a standardized concentration threshold at 0.0.14 nmol/L was the most appropriate discriminator for non-adherence (not shown in figure). After stopping drug intake, the estimated time to reach this threshold was median 3.8 days (range 2.2 to 14 days) for the 10 mg dose, 4.3 days (2.8-16) for the 20 mg dose, 5.0 days (3.3-18) for the 40 mg dose, and 5.6 days (3.8-20) for the 80 mg dose.

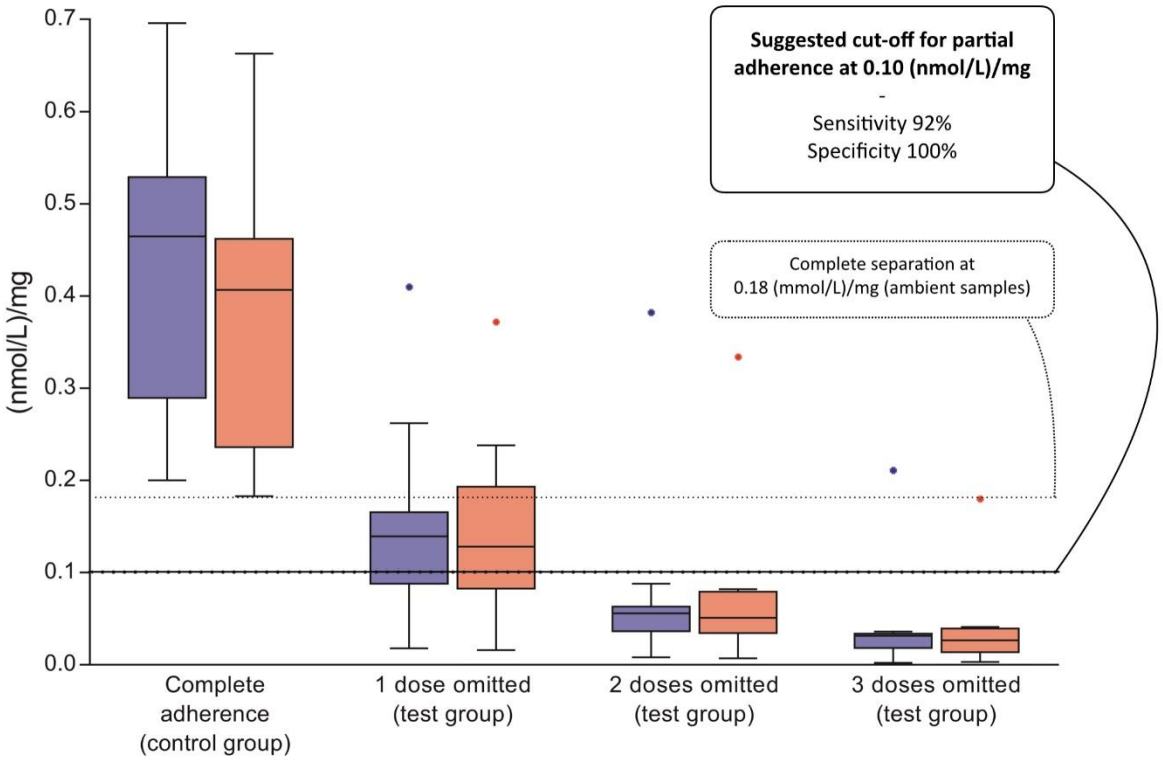

Figure 5 - Tukey box-and-whisker plots of dose-normalized concentration sums of atorvastatin acid and five metabolites (nM/mg) in the completely adherent control group and the test group omitting drug intake over a period of 3 days. Blue boxes represent samples handled in low temperature and red boxes ambient temperature.

4.2 Paper 2

Key baseline characteristics of all participants are provided in table 2. The direct method classified 344 (92.2%) participants as adherent whereas 29 (7.8%) participants were classified with reduced adherence. In patients classified as adherent by the direct method, 21% (*n*=73) reported side effects attributed to their cardiovascular drugs, whereas 41% (*n*=12) classified with reduced adherence reported such side effects (*p*=0.007). Otherwise, there were no differences in clinical, sociodemographic or psychological characteristics between these groups. Overall agreement between the direct method and self-reported adherence was fair to moderate (Figure 6).

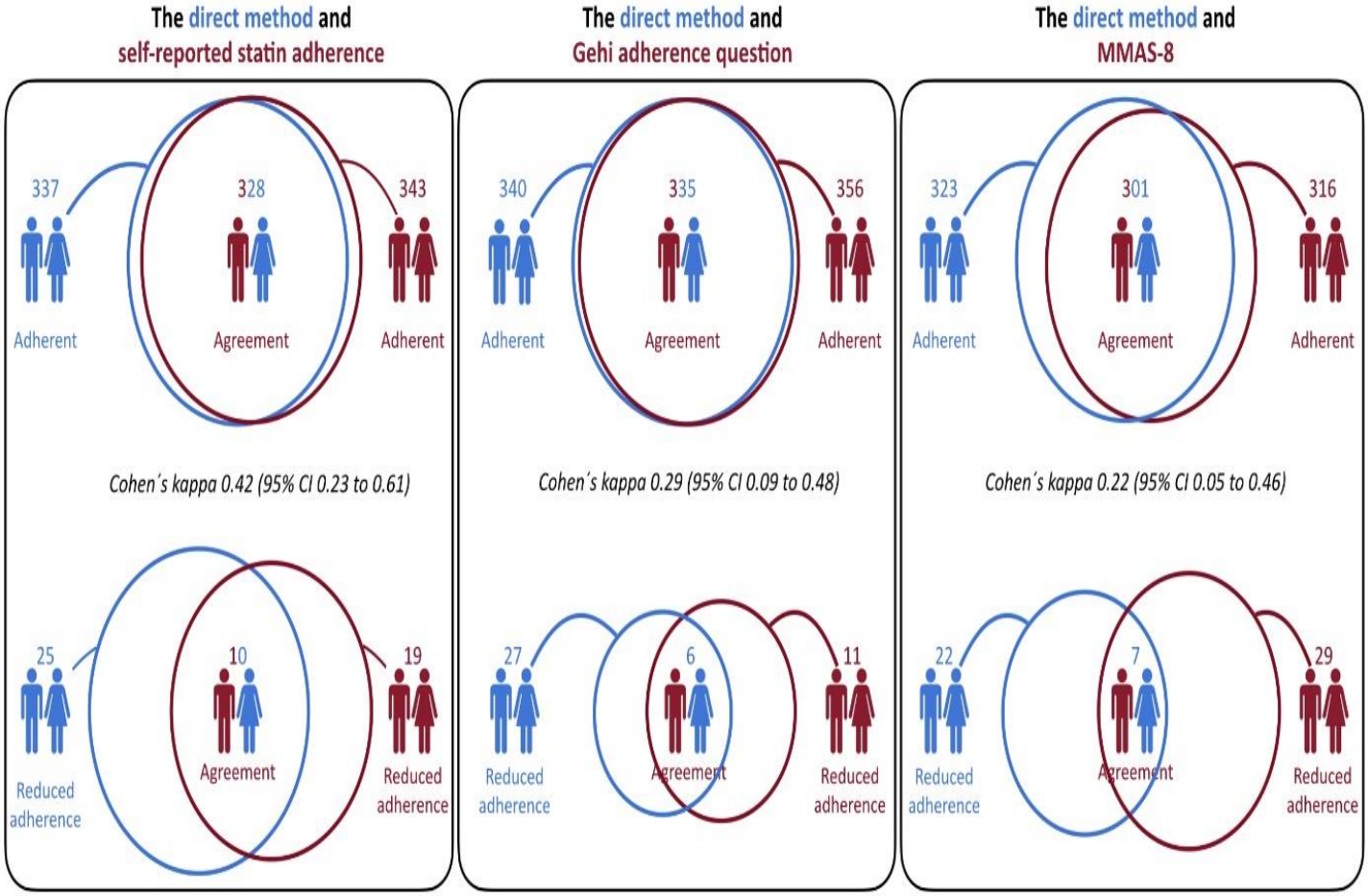

Figure 6 – Agreement between directly measured adherence to atorvastatin and self-report adherence measures in Paper 2. Blue colors represents the direct method, red colors represents the self-report measures.

In participants classified with reduced adherence by the direct method, 40%, 32% and 22% were also classified with reduced adherence by the statin adherence question, MMAS-8 and the Gehi et al.

question, respectively. For participants classified as adherent by the direct method, 96%, 95% and 94% reported accordingly on the statin adherence question, MMAS-8 and the Gehi et al. question.

Blood LDL-C concentrations were higher in patients with reduced adherence (mean 2.8 mmol/L [SD 1.0]) compared to those classified as adherent (mean 1.9 mmol/L [SD 0.6]) by the direct method (p<0.001). Participants self-reporting reduced adherence to statin therapy the past seven days (n=19, 5.5%) had higher LDL-C than those reporting to be adherent (mean 2.8 mmol/L [SD 1.0] vs. 1.9 mmol/L [SD 1.0], p=0.001). For the Gehi et al. adherence question, participants reporting reduced adherence to medication (n=11, 3%) had higher LDL-C than those reporting to be adherent (mean 3.2 mmol/L [SD 1.1] vs. 1.9 mmol/L [SD 1.0], p=0.004). LDL-C in participants classified with reduced adherence by MMAS-8 was not different from those classified as adherent (mean 2.1 mmol/L [SD 0.8] vs. 1.9 mmol/L [SD 1.0], p=0.07).

The non-participants (rest of the NOR-COR cohort) had a less favorable cardiovascular risk profile including poorer LDL-C control, higher hs-CRP and more comorbidities than the study participants (Table 5).

Table 5 – Key baseline characteristics of non-participants and participants in the present sub-study.

Characteristic	**Non-participants (N=754, 66.9%)**	**Present sub-study (N=373, 33.1%)**	***p*-value**
Sociodemographic factors			
Female, n (%)	169 (22.4)	70 (18.8)	0.105
Age in years, mean (SD)	*62.0 (9.8)*	60.9 (9.2)	0.054
Clinical factors			
Acute myocardial infarction, n (%)	573 (76.0)	321 (86.1)	<0.001
>1 coronary event prior to the index event, n (%)	254 (33.7)	85 (22.8)	0.001
LDL-C in mmol/L, mean (SD)	2.2 (0.8)	1.9 (0.7)	0.001
Charlston comorbidity score, mean (SD)	4.2 (1.5)	3.9 (1.3)	0.006

C-reactive protein, median (IQR)	1.7 (0.9-3.0)	1.4 (0.6-2.7)	0.010
Diabetes, n (%)	149 (19.8)	43 (11.5)	<0.001
Physical activity <1 time per week, n (%)	146 (19.8)	52 (13.9)	0.023
Psychological factors			
HADS-A, mean (SD)	4.8 (3.8)	4.7 (3.7)	0.833
HADS-D, mean (SD)	4.0 (3.3)	3.6 (3.1)	0.052
Bergen insomnia scale, mean (SD)	14.2 (11.0)	13.4 (10.4)	0.281
Type D social inhibition score, mean (SD)	7.5 (5.6)	7.7 (5.7)	0.432
Type D negative affectivity score, mean (SD)	7.1 (5.9)	6.8 (5.9)	0.459

HADS-A: Hospital Anxiety and Depression scale – anxiety subscale; HADS-D: Hospital Anxiety and Depression scale – depression subscale

4.3 Paper 3

2272 consecutive hospital medical records were screened to identify 982 possibly eligible CHD patients. These patients were screened for SAMS through invitation letters and telephone interviews. All participants reporting previous or ongoing muscle symptoms attributed to atorvastatin treatment during telephone interviews were invited to baseline (*n*=107). Ninety-seven patients (9.9%) still reported SAMS at baseline and 77 were randomized. Seventy-one participants completed the trial. The participant flow-chart is shown in Figure 2.

Adherence to allocated intervention was confirmed by pills counts (99% of pills removed from containers) and by the direct method (one patient excluded from per protocol analyses due to atorvastatin present in blood during placebo period). Table 6 shows balanced key baseline characteristics by randomized treatment sequences among participants who completed the trial.

Table 6 – Key baseline characteristics of participants according to randomized treatment sequence in Paper 3

Characteristic	Atorvastatin → placebo N=36, (50.7%)	Placebo → atorvastatin N=35 (49.3%)	Total (N=71)
Demographics			
Age (years), mean, (SD)	63.8 (7.8)	63.1 (11.0)	63.5 (9.5)
Female, n (%)	12 (33.3)	11 (31.4)	23 (32.4)
Low education[a], n (%)	21 (58.3)	24 (68.6)	45 (63.4)
Index coronary diagnosis			
Myocardial infarction, n (%)	30 (83.3)	30 (85.7)	60 (84.5)
Time since last coronary event, months, mean (SD)	25.0 (16.4)	20.4 (10.0)	22.7 (13.7)
Statin treatment and history of intolerance			
Previous atorvastatin discontinuation due to side effects, n (%)	13 (36.1)	13 (37.1)	26 (36.6)
Moderate or low intensity statin therapy[b], n (%)	19 (52.8)	12 (34.3)	31 (43.7)
No ongoing statin therapy, n (%)	5 (13.9)	3 (8.6)	8 (11.3)
Total number of statins used previously, mean (SD)	1.36 (0.64)	1.31 (0.58)	1.34 (0.61)
Used 2 different statins previously, n (%)	7 (19.4)	7 (20.0)	14 (19.7)
Used 3 different statins previously, n (%)	3 (8.3)	2 (5.7)	5 (7.0)
Laboratory tests			
Low density lipoprotein cholesterol (mmol/L), mean (SD)	2.50 (1.19)	2.29 (0.85)	2.40 (1.03)
Creatine kinase (U/L), mean (SD)	136 (99)	146 (94)	141 (96)
Alanine aminotransferase (U/L), mean (SD)	34.8 (16.5)	35.5 (23.8)	35.1 (20.3)
High-sensitivity C-Reactive Protein (mg/L), mean (SD)	3.62 (8.08)	2.39 (0.85)	3.01 (6.03)
Comorbidities			
>1 previous coronary event, n (%)	10 (27.8)	16 (45.7)	26 (36.6)
Heart failure, n (%)	8 (22.2)	6 (17.1)	12 (16.9)
Arthrosis, n (%)	15 (41.7)	10 (32.3)	25 (37.3)

Concomitant medication used regularly			
Total number of concomitant drugs, n (SD)	5.3 (2.3)	5.5 (1.9)	5.4 (2.1)
NSAIDs or analgesics, n (%)	7 (19.4)	5 (14.3)	12 (16.9)

NSAIDS: Non-steroidal anti-inflammatory drugs

[a]Low education was defined by completion of primary and secondary school only.

[b]High-intensity statin therapy means drug regimens that are known to lower LDL cholesterol on average by approximately 50%: (i.e. ≥40 mg atorvastatin/day or ≥20 mg rosuvastatin/day). All the other drug regimens were considered as low- or moderate-intensity statin treatment.

Atorvastatin did not affect the intensity of muscle symptoms (Figure 7). Among the 71 patients who completed the trial 20 (28%) had more symptoms during treatment with atorvastatin (confirmed SAMS), 39 (55%) had similar symptom intensity during both periods, and 12 (17%) had more muscle symptoms during the placebo period.

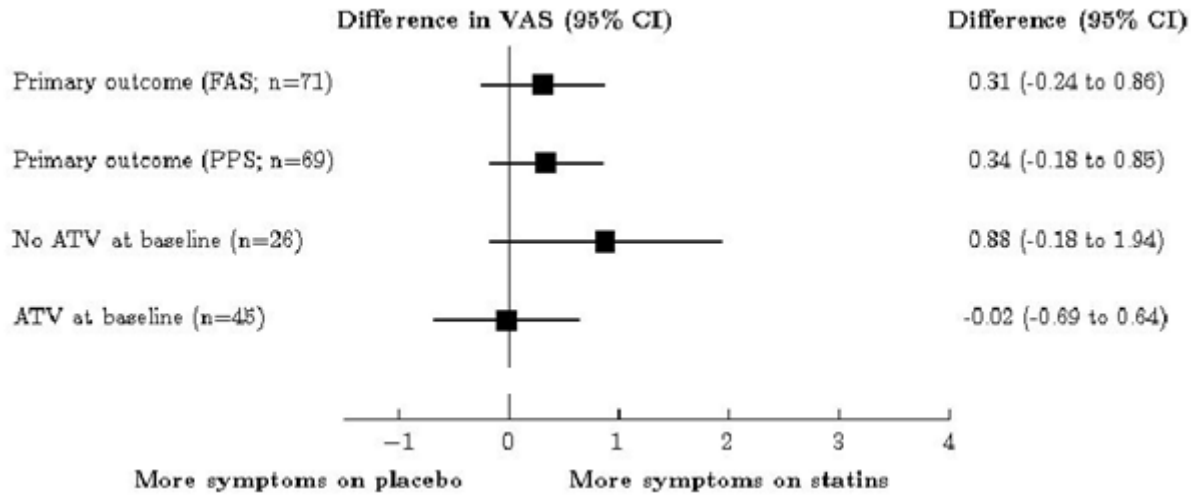

Figure 7 – Effect of atorvastatin on muscle symptom intensity in Paper 3. ATV, atorvastatin; FAS, full analysis set, PPS, per-protocol set; SAMS, statin-associated muscle symptoms.

Among participants with confirmed SAMS, there were no correlation between muscle symptom intensity and blood plasma concentrations of atorvastatin and/or metabolites (Spearmans rho ≤0.4 for all. Clinical and pharmacogentic characteristics did not differ among participants

classified with confirmed SAMS, non-SAMS and the control group without any history of muscle symptoms (Table 7).

Table 7 - Characteristics of participants in the crossover trial and the control group in Paper 3

Characteristic	**Confirmed SAMS** *n*=20 (28.1%)	**Non-SAMS** *n*=51 (71.8%)	**Control group** *n*=40
Baseline characteristics			
Women, n (%)	7 (35.0)	16 (31.4)	12 (30.0)
Age (years), mean (SD)	64.1 (11.0)	63.2 (8.9)	64.2 (8.6)
Previous atorvastatin discontinuation, n (%)	8 (40.0)	28 (54.9)	0 (0)
High intensity statin at baseline, n (%)	12 (60.0)	28 (54.9)	38 (95.0)
Body mass index (kg/m^2), mean (SD)	27.6 (4.1)	28.5 (4.4)	28.3 (4.1)
High physical activity, n (%)	12 (60.0)	27 (54.0)	23 (57.5)
Alanine aminotransferase (U/L), mean (SD)	30.7 (14.9)	36.9 (21.9)	41.6 (23.4)
Creatinine (µmol/L)	84.7 (40.6)	81.9 (16.5)	82.3 (35.6)
Estimated GFR (ml/min/1.73m^2), mean (SD)	78.7 (18.2)	78.5 (12.8)	78.6 (17.4)
Total number of concomitant drugs, n (SD)	5.9 (2.5)	5.2 (1.9)	5.3 (1.6)
Regular use of analgesics, n (%)	4 (20.0)	11 (21.6)	3 (7.5)
*CYP3A4 *1/*1, n (%)*	17 (85.0)	47 (92.2)	37 (92.5)
*CYP3A4 *1/*22, n (%)*	3 (15.0)	4 (7.8)	3 (7.5)
*CYP3A4 *22/*22, n (%)*	0 (0)	0 (0)	0 (0)
*CYP3A5 *1/*1, n (%)*	0 (0)	0 (0)	0 (0)
*CYP3A5 *1/*3, n (%)*	3 (15.0)	6 (11.8)	7 (17.5)
*CYP3A5 *3/*3, n (%)*	17 (85.0)	45 (88.2)	33 (82.5)
*SLCO1B1 *1/*1, n (%)*	17 (85.0)	37 (72.6)	26 (65.0)
*SLCO1B1 *1/*5, n (%)*	3 (15.0)	14 (27.5)	13 (32.5)
*SLCO1B1 *5/*5, N (%)*	0 (0)	0 (0)	1 (2.5)
Characteristics during the treatment period on atorvastatin			

Alanine aminotransferase (U/L), mean (SD)	29.9 (14.4)[a]	33.5 (17.1)	45.0 (59.7)
Creatine Kinase (U/L), mean (SD)	102 (41.1)	152 (83.6)	128 (77.6)
Lactate Dehydrogenase (mmol/L), mean (SD)	165 (35.0)	180 (37.2)	181 (28.3)

SD: standard deviation; nM: nano-mol; C: concentration; CI: confidence interval

[a]One patient with an adverse reaction (i.e. elevation of alanine aminotransferase >10 x upper normal limit) at the end of the atorvastatin treatment period was excluded from this analysis.

5. Discussion

5.1 Summary of main findings

Poor statin adherence represent a major public health concern in the prevention of CHD. Valid methods for measuring adherence and more knowledge of statin side effects are of utmost importance to quantify, understand and address this challenge. The present PhD-project has developed a novel way of directly measuring adherence to atorvastatin, the most commonly used statin, based on blood concentrations of the parent drug and its major metabolites. We have shown that the direct method may classify patients as adherent, partially adherent or non-adherent with high sensitivity and specificity.

Applying this method in a cohort of 373 Norwegian CHD patients, we have shown that only 22-40% of patients detected as having reduced adherence with the direct method, responded correspondingly on anonymous self-report questionnaires. On the other hand, 90% of the participants that were tested adherent by the novel direct method, self-reported being adherent to their statin in the past seven days.

Finally, we estimated a prevalence of 10% SAMS in an outpatient CHD population. In a crossover RCT, atorvastatin 40 mg did not affect the intensity of muscle symptoms in these patients. There were no correlation between levels of atorvastatin in blood and muscle symptoms on the subgroup classified with confirmed SAMS. Altogether, these results suggest that muscle symptoms have causes other than the statin in most patients.

5.2 Directly measuring statin adherence

Given valid assays and criteria to translate the outcome into terms of adherence, measurement of drug blood concentrations provides proof of actual drug intake and thereby eliminate the most important limitations of indirect measures (Table 1). For atorvastatin, several assays have been described (91-93), and employed to determine adherence (93, 94). However, to

interpret these results, an understanding of measured concentrations in relation to drug intake is required. In paper one, we estimated that atorvastatin and/or metabolites are detected for several weeks after the last dose had been ingested. Classifying adherence by presence or absence of the drug in blood, solely, is therefore likely to provide suboptimal sensitivity in a clinical setting given the high detection sensitivity of modern LC-MS/MS and the half-life of atorvastatin. Further, as the limit of detection will vary between and within laboratories, such a dichotomous approach has limitations concerning standardization, as the limit of detection will vary between laboratories. The method developed in paper one takes direct measurement of atorvastatin adherence a step further, allowing a more fine-tuned assessment of adherence that relates to a number of days without drug intake. It is uncertain to what extent differentiation between partial adherence (2 or 3 days omitted) and non-adherence (>3 days omitted) represent a clinically meaningful entity, and whether the suggested cut-offs reflect long-term adherence. We did, however, find that partially adherent patients had significantly higher concentrations of LDL-C (2.4 mmol/L) from those classified as adherent (1.9 mmol/L), and significantly lower than or non-adherent patients (3.6 mmol/L) in paper two. In a recent study, Rodriquez et al. applied pharmacy refill data to measure statin adherence (medication possession ratio, MPR) in 347 000 patients with CHD (29). They demonstrated a graded relationship between statin adherence and all-cause mortality: In patients with MPR <50% the hazard ratio (HR) was 1.30, in those with MPR 50% to 69% the HR was 1.21, and even in patients with MPRs up 70% to 89%, the hazard ratio for all-cause mortality was 1.08 (95% CI 1.06 to 1.09). This underlines the clinical relevance of fine-tuned methods when using blood concentrations for measuring statin adherence (29). In the study by Rodriguez, efforts were made to adjust for overall healthy behavior. Nonetheless, the healthy adherer effect cannot be fully excluded (95, 96).

Although adherence measured by self-report questionnaires reflect dispensing data and predict CVD events, they tend to overestimate drug intake when compared to more robust measures (86, 96-99). We found that only 22%-40% of those with atorvastatin blood concentrations suggestive of reduced adherence self-reported accordingly, underlining the fact that self-report is prone to both

recall and reporting bias and do not prove actual drug intake. Similarly, Bergland et al. reported poor overlap between self-report data and directly measured adherence to antihypertensive medications (100). Along these lines, a recent state of the art review advised against using self-report as the sole measure of adherence in future studies (41). Indeed, the most recent ESC/ESH guidelines on hypertension from 2018 recommend to measure drug concentrations in blood or urine in patients with apparent treatment-resistant hypertension (101).

5.3 Estimated prevalence of reduced adherence and potential correlates

The estimated prevalence (8%) of reduced adherence to atorvastatin in our study was much lower than estimates from outside Norway were 20%-50% of patients are found to be non-adherent after a CHD event (35, 36). However, comparisons are difficult due to societal differences as well as differences in healthcare systems and measurement methods. Medication costs are known to influence statin adherence (48, 102). In Norway, CHD patients only pay a deductible amounting to approximately 35 USD/year, thereby largely removing the economic adherence barrier (103). In the recent nationwide study from Norway by Bergland et al, 7.3% of patients were found to be non-adherent to their antihypertensive medication based on measurement of drug concentrations with predefined cut-offs (100), much like in our study. Similarly, another Norwegian study by Halvorsen et al. reported a 6% drop in the proportion of patients being prescribed a statin within the first year after a CHD event (40). Although the study by Halvorsen et al. does not indicate to what extent the pills are taken by the patients, it supports a notion of relatively high adherence to medication in Norwegian CHD patients.

Mental health issues and personality traits are associated with poor adherence. In particular, depression and type D personality has been associated with poor adherence to CHD medications in several studies (32, 85, 104, 105). This association has not previously been explored in a setting of directly measured statin adherence as reported in paper two. Albeit a small non-significant numerical trend towards more depressive symptoms in patients with reduced adherence (HADS-D 5.2 (reduced

adherence) vs. 4.7 (adherent), these results are within the normal range indicating a low symptom burden in our sample. Further, lack of power did not allow us to dichotomize at clinically relevant cut-offs (HADS ≥8). The same applies to the insomnia scale and the type D subscales. Patient selection could possibly explain these results. The scores of the participants in this sub study did not differ from the rest of the total NOR-COR cohort. Hence, our results do not undermine the importance of the established association between psychological factors and poor adherence.

We found that drug-related side effects were reported in a higher proportion of participants classified with reduced adherence than those who were adherent (41% vs 21%, *p*=0.007). Although we did not enquire about statin side effects specifically, it is likely that a significant proportion of their side effects are statin-related. In a large US survey of 10 000 former and current statin users, muscle symptoms were reported by 60% and 25%, respectively (67), and a large Swedish RCT recently reported side effects as being the most common reason for statin discontinuation in CHD patients (106) .

5.4 Muscle side effects of statins

Ten percent of the CHD patients screened for participation in the MUSE trial reported muscle symptoms self-perceived as caused by atorvastatin. This reflects the frequency of which health care providers face patients with SAMS and our estimated prevalence is the same as reported in the large PRIMO survey from France (66). In a recent meta-analysis including >4 000 000 patients, the overall prevalence of statin intolerance was 9.1% (17% in cohort studies and 5% in RCTs). When formal diagnostic criteria were applied, the overall prevalence was 6% to 7% (107). MUSE participants were not selected based on formal diagnostic algorithms for SAMS. Indeed, upon review of hospital medical records we could not find that systematic re-challenge had been conducted in any of the participants. This may increase the generalizability of our results, as one can argue that these are the patients faced in routine clinical practice.

Overall, we found no effect of atorvastatin on the intensity of muscle symptoms. Although 28% of participants fulfilled our predefined definition of confirmed SAMS, 17% reported more symptoms on placebo than atorvastatin, indicating a nocebo effect. After trial completion, we presented the study results to all participants and provided tailored advice regarding further lipid lowering treatment. After 13 months follow-up, 91% were able to tolerate a statin including 16 of the 20 participants classified with confirmed SAMS (108). Two N-of-1 trials from the UK studying the effect of atorvastatin 20 mg/day have recently reported similar results (109, 110). One of these trials also included periods without tablets at all as well as periods with statin and placebo, allowing the authors to conclude that 90% of the participants' symptoms were due to the nocebo effect (111). Even in highly selected and perceived statin-intolerant patients recruited to test proprotein convertase subtilisin/kexin type 9 inhibitors, the vast majority can indeed tolerate blinded atorvastatin treatment (112). Altogether, it seems that for most patients, muscle side effects of atorvastatin are caused by the act of taking a pill rather than the by its content.

By introducing a negative expectation of side effects, Tinnermann et al. have demonstrated a physiological rationale for the nocebo effect (113). In an experiment, they showed that labelling an inert medication as "expensive" produced more side effects as compared to a labelling it as "cheap", despite the content being identical. Functional magnetic resonance imaging during the experiment revealed differences in brain activity in areas involved in pain processing (113).

5.5 Muscle side effects and systemic atorvastatin exposure

Despite several previous studies have suggested a connection between muscle symptoms and blood concentrations of atorvastatin and metabolites (61), in particular the lactone forms (62, 63, 114), we found no such relationship in MUSE. Neither trough nor peak concentrations correlated with the intensity of muscle symptoms in patients classified with confirmed SAMS. A stringent and blinded test procedure allowed evaluation of the causal relationship between the drug and the symptoms. Although MUSE could not verify the existence of a small sub-group with truly statin-dependent

symptoms, such a population likely exists (115). By selecting participants based on previous statin discontinuation or subjective intolerance to a certain number of statins, we would likely have increased the proportion of patients with truly statin-dependent symptoms and thereby statistical power. Nonetheless, it remains uncertain whether the plasma concentration of atorvastatin and metabolites reflect the local drug exposure in muscle tissue.

6. Limitations

6.1 General limitations

Several methodological aspects should be considered when interpreting the results of this thesis. In the following, the most important limitations are elaborated.

First, all study participants had established CHD. The results are therefore not outright representative of other patient categories. For example, statin adherence is apparently poorer in a setting of primary prevention than in secondary prevention (116). Furthermore, the vast majority of participants were Caucasian. This needs consideration when applying the results to populations of other decent as they differ in important characteristics affecting the pharmacokinetics of atorvastatin (117). This may influence classification of adherence as well as susceptibility to side effects. Further, we only studied atorvastatin. Other statins may entail a higher or lower risk of muscle side effects. Altogether, these limitations reduce the generalizability (i.e. external validity) of the results.

Second, we used the same LC-MS/MS assay for measuring atorvastatin and metabolites across the studies. Although considered the gold standard for measuring drugs and metabolites in blood, the LC-MS/MS assay has some random uncertainties. During method validation, the coefficients of variation (CVs) were maximum 8.1% across the measurement ranges for all six analytes. Carryover from one sample to the next and interferences from other components are potential sources of error. These methodological aspects have been evaluated and found to be equal to zero in the present method. Ion suppression (i.e. reduction of instrument signals caused by plasma components) was observed for three of the analytes. These matrix effects were corrected by applying isotope-labeled internal standards in each sample (55). To ensure correct calibrator levels, the declared degree of purity of the substances was used in the calculation of the solutions' concentrations. The calibrator solids were either purchased in exact weight from the manufacturer, or they were weighted in-house using a weight that was quality assured according to the ISO15189

standard. Despite the abovementioned precautions, systematic measurement error cannot be entirely excluded. The same personnel used the same instruments and conducted all LC-MS/MS analyses at the same laboratory. Hence, there is likely a very low variation between the measurements in the different studies. However, there may still be limitations with regards the generalizability of measured concentrations as we did not attempt to compare our measurements to other laboratories performing the same analyses (e.g. proficiency testing scheme).

Third, the number of study participants were relatively low. In paper one, the purpose of the study was to establish detection limits, and the number of participants was decided accordingly. Paper two is a post-hoc analysis, the number of participants were therefore predefined. Hence, for paper one and two, there were no a priori power calculations and the results from statistical analyses should be considered explorative. With low statistical power, there is a risk of rejecting a null hypothesis when it is in fact true (i.e. finding an effect when there actually is none – type 1 error), as well as not rejecting a null hypothesis when it is in fact false (i.e. not finding an effect when there actually is one - type 2 error). Small sample size does not allow adjusted statistical analyses, and advocates caution upon conducting and interpreting hypothesis testing. With a larger number of participants we could have explored factors affecting blood concentrations of atorvastatin in paper one, potentially modifiable factors associated with reduced adherence in paper two, and possibly more patients with confirmed SAMS would have been found in paper three. In paper three, the study was powered for the primary endpoint. Hypothesis testing beyond the primary endpoint are there considered explorative. For example, the subgroup who were not using atorvastatin at baseline (N=26, Figure 7) had a lower bound of the 95% CI at -0.18 and an upper bound at 1.94. The difference is therefore not statistically significant. It is possible that this represent a type 2 error as a larger sample size would produce a narrower confidence interval and potentially statistically significant difference.

Finally, we plotted all study data manually into SPSS for management and analyses. Although two persons conducted this together and random sampling by a third person checked quality, there is a risk that errors may have occurred. This would, however, likely represent random error and thus not bias the results.

6.2 Study specific limitations

6.2.1 Paper 1

The number of patients on each dose level was low. Hence, the cut-offs may not fully address pharmacokinetic variability (i.e. genetic sequence variants and interacting drugs). Along these lines, the metabolite concentrations were apparently extremely high in a single patient. We were unable to conclude whether this represent failure to comply with study protocol or true pharmacokinetic variation. Hence, we cannot exclude the possibility that some patients with extremely high or low dose-normalized blood concentrations may be misclassified with our algorithm.

Participants were followed for three days without atorvastatin intake. Therefore, we only have empirical data on plasma concentrations for patients skipping three consecutive doses. Further, concentrations beyond three days were extrapolated based on pharmacokinetic simulation, which brings along uncertainty as compared to empirical data. To what extent our cut-offs reflect other forms of reduced adherence (i.e. implementation issues) is unknown.

The problem of escape dosing is not fully addressed as we did not simulate escape intake at other time intervals than 2 hours before blood sampling. A myriad of possibilities for escape dosing will exist in clinical practice.

The suggested cut-off values are dose-normalized as the drug dose was highly correlated to measured concentrations of atorvastatin and metabolites. By using the sum of all metabolites, pharmacokinetic variation may be accounted for to some extent. However, atorvastatin blood concentrations vary significantly among patients taking the same dose, and it is therefore possible

that our cut-off values should be adjusted for additional patient factors (for example pharmacogenetic variants) upon validation in a larger sample (118).

6.2.2 Paper 2

The present study was a single-center study as only patients from cohort Vestfold had bio-banked blood samples for direct adherence testing. This is particularly important concerning the estimated prevalence of reduced adherence. The non-participants (rest of the NOR-COR cohort) differed in important clinical and biochemical characteristics related to poor statin adherence, such as LDL-C, CRP and comorbidities. Vestfold Hospital also has a well-developed cardiac rehabilitation program with close follow-up of CHD patients. It is therefore likely that the non-participants would include a higher proportion of patients with reduced adherence. Furthermore, adherence was measured median 16 months after the index CHD event. As poor statin adherence is associated with poor prognosis (29), there is a risk of survival bias affecting the number of participants with poor adherence in our sample.

We chose to use the sum of atorvastatin and all metabolites when determining adherence. The reason for choosing this six-component sum was to account for a potentially higher pharmacokinetic variation in a larger sample than in the sample of paper one. Potentially, other metabolites or sums of metabolites (e.g. atorvastatin acid plus lactone) could be superior to classify adherence.

The relationship between adherence and LDL-C is based on a spot measurement of LDL-C. Ideally, we would assess atorvastatin and metabolites in relation to either absolute or relative change in LDL-C, as well as a spot measurement. This would have allowed evaluation of the effect of reduced adherence over time. However, this was not possible due to the lack of lipid values at the time of the index event in many study participants.

The self-report questionnaire used in NOR-COR, from which parts of the data for paper two was collected (table 3), was comprehensive. Its reproducibility has been evaluated in a test-retest study of 99 CHD patients showing high intra-individual reproducibility (119). Nonetheless, factors such as

the patients understanding of questions, confusion or distraction may introduce errors and affect the statistical associations (120).

6.2.3 Paper 3

If well conducted, double-blinded RCTs provide high internal validity and are considered the gold standard methodology in clinical research. The observed effects in RCTs are either caused by the study intervention tested, or by coincidence which is assessed by the hypothesis testing. This contrasts observational research where systematic differences between treatment groups (bias) frequently occur. However, the generalizability of RCTs is largely confined to patients fulfilling the trial inclusion criteria. In MUSE, we did not base inclusion criteria on proposed diagnostic algorithms for SAMS (47, 72). However, these algorithms have not previously been validated against a gold standard for diagnosing SAMS (i.e. blinded crossover study) and were not routinely used in clinical practice at the time of planning this study. On one hand, the use of these algorithms as formal inclusion criteria would likely have decreased the number of patients with symptoms with other causes than atorvastatin (107). On the other hand, their use could have contributed to poorer generalizability to routine clinical practice. Along these lines, it is noteworthy that 16 out of 19 patients with previous intolerance to two or more statins were classified as non-SAMS in MUSE. We excluded patients with a short life expectancy. These are older, have more comorbidities and concomitant medications and therefore an increased likelihood of experiencing side effects.

The MUSE trial tested the effect of atorvastatin on muscle symptoms only. Even though muscle symptoms is by far the most common side effect of statins, other statin side effects were not evaluated.

The MUSE trial included only one crossover. With several crossovers, the possibility of random fluctuations in muscle symptoms affecting our results would have been minimized and our results strengthened. In an early phase, we planned MUSE with two crossovers, but due to practical issues of study implementation and costs, a single crossover was chosen. Given the results of recent

N-of-1 trials with several crossovers, it is likely that even fewer patients with confirmed-SAMS would have been found. We did inform the participants only to report muscle symptoms that they had previously experienced and attributed to statin intake. Our conclusion that only a minority of patients confronting physicians with SAMS truly have statin-dependent symptoms, is therefore nonetheless valid.

The study participants reported their muscle symptoms in a diary. This may introduce reporting bias as participants may have consulted previous results upon reporting, as opposed to being blind to previously reported symptom intensity. This is of particular importance if participants had compared their results from the previous treatment period. To reduce this risk, we therefore collected the diaries between the treatment periods.

We measured atorvastatin and metabolites immediately prior to the scheduled dose and two hours after ingestion of atorvastatin. This yields the trough and peak concentrations, but does not reflect the exposure throughout the entire dosing interval. It is possible that the total exposure would have correlated better with the intensity of muscle symptoms and therefore be better suited to discriminate patients with truly statin-dependent symptoms. Blood sampling several times each study day, however, would have markedly limited participation and hence the generalizability.

7. Ethical considerations

All studies were conducted in accordance with the ethical principles of the Declaration of Helsinki and in consistence with ICH/Good Clinical Practice. All patients gave written informed consent prior to study start. The study protocol for paper one was reviewed by the Regional Committee for Medical and Health Research Ethics and The Norwegian Medicines Agency. However, they did not define the study as a study requiring approval as they did not define it as health research aiming to ascertain or verify/compare the efficacy or safety of atorvastatin. The study was approved by the local Data Protection Officer (16/00117–107). The study protocol for paper two was approved by the Regional Committee for Medical and Health Research Ethics (2013/1885). Since study data was collected before July 2018, approval from the local Data Protection Officer was not required. The MUSE trial (paper three) was reported according to the CONSORT guidelines and registered in the European Clinical Trials Database (2018-004261-14) and at ClinicalTrials.gov (NCT03874156), prior to inclusion of the first patient. The trial was approved by the Regional Committees for Medical Research Ethics (2018/2302), the Norwegian Medicines Agency (18/17102-16), and the local data protection officers (16/00117-137). The trial was monitored by research cardiologists.

Except for a comprehensive and demanding study protocol with repeated blood sampling, I am not aware of important ethical issues in paper 1. The potential risk of adverse cardiovascular events during the period without statin treatment is very low (121) .

Ethical issues in paper two relates to results from the clinical examination in NOR-COR that required further medical attention such as elevated blood pressure, HbA1c or LDL-C values. The study cardiologist or the primary care physician followed up participants with such findings to ensure that appropriate treatment was initiated. Another issue relates to the use of blood samples to reveal statin non-adherence without specifically informing the study participants. This was regarded a necessity to eliminate white coat adherence and all participants consented that their bio-banked blood could be used in future research. Since the study was conducted almost five years prior to the

statin analyses, we decided to not follow-up the results by attempting to contact the participants or their treating physician.

There are certain ethical issues in paper three that deserve consideration. First, the study protocol was comprehensive and demanding with repeated blood samples. Second, there is a potential risk of adverse cardiovascular events during the 8-week period without statin treatment and a risk of serious side effects (e.g. acute liver failure, rhabdomyolysis, myopathy) during treatment with atorvastatin. Two minimize the risk of myopathy or acute liver failure; we excluded patients with a history of these conditions and monitored CK and ALT during the trial. Two previous randomized studies have investigated the risk of adverse CVD events during a short time without statin therapy in CHD patients. Heeschen and colleagues reported a trend toward greater risk of death and nonfatal MI when statins therapy was withdrawn after an admission for an acute coronary syndrome (122). Importantly, all MUSE participants were included at least six months after their last CHD event and were thoroughly assessed for symptoms of unstable CHD prior to randomization. In another randomized study of more than 15 000 stable CHD patients, 6 weeks statin discontinuation did not lead to recurrent cardiovascular events or mortality (121). Since our study population included patients with ongoing side effects or who had discontinued their prescribed statins, they were at significantly increased risk of subsequent CVD events compared to a general CHD population. Hence, many of the MUSE participants likely received better statin therapy during the trial than prior to inclusion.

8. Conclusions

This thesis introduces a novel direct method with cut-off values allowing discrimination among adherence, partial adherence and non-adherence to atorvastatin therapy in patients with CHD (paper one). Patients omitting atorvastatin for two to three days where considered partially adherent and those omitting more than three were considered non-adherent. In paper two, the novel direct method reflected spot LDL-C levels in blood and showed fair to moderate agreement with self-reported adherence measures. The direct method revealed more patients with reduced adherence than the self-report measures. In the present sample of CHD patients, only eight were classified with reduced adherence. These patients did not differ regarding clinical and psychosocial characteristics except for side effects being associated with reduced adherence. In patients with previous or ongoing muscle complaints attributed to atorvastatin therapy, there was no effect of high-intensity atorvastatin on muscle symptom intensity upon blinded re-challenge (paper three). Blood concentrations of atorvastatin and/or metabolites were not associated with statin-dependent muscle symptoms. Although the possibility of truly statin-dependent symptoms cannot be excluded in some patients, such symptoms are much more likely to have other causes. Overall, the thesis provides novel measurement methods and extends our understanding about adherence and side effects of atorvastatin in patients with CHD. This knowledge may contribute to individualize and improve current clinical practice for treatment and follow-up care for these patients with potential beneficial long-term effects.

9. Clinical implications and future perspectives

The results from paper one and two indicate that the direct method may be a useful supplement in clinical practice to evaluate statin adherence. The method is likely feasible as the blood sampling and pre-analytical procedures are simple and familiar even outside the hospital setting. Furthermore, the cost of using the method is acceptable (Nils Tore Vethe, personal communication). Monitoring statin adherence may provide an entry point into a discussion with the patient about causes of poor adherence and barriers preventing adequate adherence. Furthermore, as patients may improve their adherence upon being made aware of the results from the adherence test, the method itself may prove valuable to improve adherence.

Importantly, the cut-offs needs to be validated in a larger sample with other comorbidities, more interacting drugs and larger pharmacogenetic diversity. We have therefore recently conducted a validation study in 60 patients treated with atorvastatin (data collection completed in late 2021). In addition, we are planning another validation study against the Norwegian prescription registry to evaluate whether spot measurements reflect long-term persistence to statin treatment. We also aim to test the clinical usefulness of the direct method in future studies. The direct method will be used to determine statin adherence and to explore clinical and psychological factors in the ongoing, national BEtablocker Treatment After Acute Myocardial Infarction in Patients Without Reduced Left Ventricular Systolic Function (BETAMI) trial (www.clinicaltrials.gov, identifier NCT03646357).

The thesis have verified that SAMS are frequently encountered in clinical practice. The main finding was that high-dose treatment with atorvastatin did not affect muscle symptom intensify in CHD outpatients. This is an important message and clinicians should therefore search for alternative causes of muscle complaints in these patients and assess the temporal association between symptoms and statin intake prior to reducing the dose, switching to less potent drug classes or discontinuing treatment. It is recommended that health care professionals spend sufficient time in order to establish a trustful relationship with the patient (123). Potential negative expectations regarding statin therapy should be managed carefully, preferably at the time of drug prescription, by

explaining the strong documentation and beneficial effects of statins as well as high tolerability as documented in the present thesis and other recent publications. Most likely, the population of patients reporting subjective SAMS include a small sub-group of who are truly statin intolerant. To elucidate pathophysiological mechanisms in these patients, we are currently conducting a study on a subset of the MUSE participants classified with statin-dependent symptoms (www.clinicaltrials.gov, identifier NCT04453735). In this study, we aim to characterize the molecular pattern in blood and muscle tissue in order to derive potentially diagnostic markers for statin-dependent muscle symptoms. Psychological distress like anxiety, depression or type D personality disorder could potentially influence the patients' perception of muscle symptoms and thus the risk of experiencing SAMS. However, in a post-hoc analysis, we recently showed that psychological factors were not associated with neither self-perceived nor confirmed SAMS (124). The nocebo effect is likely to contribute significantly to symptom burden in many patients, and communication interventions addressing the risk of side effects may prove effective in reducing the risk of patients experiencing SAMS. Last, as the treatment targets for LDL-C are rapidly lowered the effect of high dose, high intensity lipid lowering treatment on muscle symptoms should be assessed in future studies.

10. References

1. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. FEBS Lett. 1976;72(2):323-6.

2. Tsujita Y, Kuroda M, Tanzawa K, Kitano N, Endo A. Hypolipidemic effects in dogs of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Atherosclerosis. 1979;32(3):307-13.

3. Kuroda M, Tsujita Y, Tanzawa K, Endo A. Hypolipidemic effects in monkeys of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Lipids. 1979;14(6):585-9.

4. Yamamoto A, Sudo H, Endo A. Therapeutic effects of ML-236B in primary hypercholesterolemia. Atherosclerosis. 1980;35(3):259-66.

5. Endo A. A historical perspective on the discovery of statins. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86(5):484-93.

6. Endo A. Monacolin K, a new hypocholesterolemic agent produced by a Monascus species. J Antibiot (Tokyo). 1979;32(8):852-4.

7. U.S Food and Drug Administration [Web page]. www.fda.gov: U.S Food and Drug Administration; 2022 [cited 2022 March 1st]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2001/20-702S025_Lipitor.cfm.

8. Brenner GM, Stevens CW. Brenner and Stevens' Pharmacology. Fifth ed: Elsevier; 2018.

9. Amarenco P, Bogousslavsky J, Callahan A, 3rd, Goldstein LB, Hennerici M, Rudolph AE, et al. High-dose atorvastatin after stroke or transient ischemic attack. N Engl J Med. 2006;355(6):549-59.

10. Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. Lancet. 2016;388(10059):2532-61.

11. Mraiche F, Cena J, Das D, Vollrath B. Effects of statins on vascular function of endothelin-1. Br J Pharmacol. 2005;144(5):715-26.

12. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov. 2005;4(12):977-87.

13. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet. 2009;373(9670):1175-82.

14. Eto M, Kozai T, Cosentino F, Joch H, Lüscher TF. Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. Circulation. 2002;105(15):1756-9.

15. Ali FY, Armstrong PC, Dhanji AR, Tucker AT, Paul-Clark MJ, Mitchell JA, et al. Antiplatelet actions of statins and fibrates are mediated by PPARs. Arterioscler Thromb Vasc Biol. 2009;29(5):706-11.

16. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). European Heart Journal. 2019;41(1):111-88.

17. Cholesterol Treatment Trialists C, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81.

18. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78.

19. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459-72.

20. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: Developed by the Task Force for

cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical societies With the special contribution of the European Association of Preventive Cardiology (EAPC). European Heart Journal. 2021;42(34):3227-337.

21. The Norwegian Prescription Database [Web page]. www.norpd.no: Norwegian Institute of Public Health; 2020 [cited 2020 November 22nd]. Available from: http://norpd.no/Prevalens.aspx.

22. Munkhaugen J, Sverre E, Otterstad JE, Peersen K, Gjertsen E, Perk J, et al. Medical and psychosocial factors and unfavourable low-density lipoprotein cholesterol control in coronary patients. Eur J Prev Cardiol. 2017;24(9):981-9.

23. Sverre E, Peersen K, Husebye E, Gjertsen E, Gullestad L, Moum T, et al. Unfavourable risk factor control after coronary events in routine clinical practice. BMC Cardiovasc Disord. 2017;17(1):40.

24. Kotseva K, Wood D, De Bacquer D, De Backer G, Ryden L, Jennings C, et al. EUROASPIRE IV: A European Society of Cardiology survey on the lifestyle, risk factor and therapeutic management of coronary patients from 24 European countries. Eur J Prev Cardiol. 2016;23(6):636-48.

25. Munkhaugen J, Peersen K, Sverre E, Gjertsen E, Gullestad L, Dammen T, et al. The follow-up after myocardial infarction - is it good enough? Tidsskr Nor Laegeforen. 2018;138(5).

26. Sabate E. Adherence to long-term therapies - Evidence for action www.who.int: World Health Organization; 2003 [Available from: https://www.who.int/chp/knowledge/publications/adherence_full_report.pdf.

27. Chowdhury R, Khan H, Heydon E, Shroufi A, Fahimi S, Moore C, et al. Adherence to cardiovascular therapy: a meta-analysis of prevalence and clinical consequences. Eur Heart J. 2013;34(38):2940-8.

28. De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. Br J Clin Pharmacol. 2014;78(4):684-98.

29. Rodriguez F, Maron DJ, Knowles JW, Virani SS, Lin S, Heidenreich PA. Association of Statin Adherence With Mortality in Patients With Atherosclerotic Cardiovascular Disease. JAMA Cardiol. 2019;4(3):206-13.

30. Cutler RL, Fernandez-Llimos F, Frommer M, Benrimoj C, Garcia-Cardenas V. Economic impact of medication non-adherence by disease groups: a systematic review. BMJ Open. 2018;8(1):e016982.

31. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med. 2005;353(5):487-97.

32. Hope HF, Binkley GM, Fenton S, Kitas GD, Verstappen SMM, Symmons DPM. Systematic review of the predictors of statin adherence for the primary prevention of cardiovascular disease. PLoS One. 2019;14(1):e0201196.

33. Hirsh BJ, Smilowitz NR, Rosenson RS, Fuster V, Sperling LS. Utilization of and Adherence to Guideline-Recommended Lipid-Lowering Therapy After Acute Coronary Syndrome: Opportunities for Improvement. J Am Coll Cardiol. 2015;66(2):184-92.

34. Kolandaivelu K, Leiden BB, O'Gara PT, Bhatt DL. Non-adherence to cardiovascular medications. European Heart Journal. 2014;35(46):3267-76.

35. Foody JM, Joyce AT, Rudolph AE, Liu LZ, Benner JS. Persistence of atorvastatin and simvastatin among patients with and without prior cardiovascular diseases: a US managed care study. Curr Med Res Opin. 2008;24(7):1987-2000.

36. Ho PM, Spertus JA, Masoudi FA, Reid KJ, Peterson ED, Magid DJ, et al. Impact of medication therapy discontinuation on mortality after myocardial infarction. Arch Intern Med. 2006;166(17):1842-7.

37. Muntner P, Yun H, Sharma P, Delzell E, Kent ST, Kilgore ML, et al. Ability of low antihypertensive medication adherence to predict statin discontinuation and low statin adherence in patients initiating treatment after a coronary event. Am J Cardiol. 2014;114(6):826-31.

38. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005;352(14):1425-35.

39. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med. 2015;372(25):2387-97.

40. Halvorsen S, Jortveit J, Hasvold P, Thuresson M, Øie E. Initiation of and long-term adherence to secondary preventive drugs after acute myocardial infarction. BMC Cardiovasc Disord. 2016;16:115.

41. Simon ST, Kini V, Levy AE, Ho PM. Medication adherence in cardiovascular medicine. Bmj. 2021;374:n1493.

42. Anghel LA, Farcas AM, Oprean RN. An overview of the common methods used to measure treatment adherence. Med Pharm Rep. 2019;92(2):117-22.

43. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2018.

44. Karlson BW, Wiklund O, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: results from VOYAGER. Eur Heart J Cardiovasc Pharmacother. 2016;2(4):212-7.

45. Bacquer D, Smedt D, Reiner Ž, Tokgözoğlu L, Clays E, Kotseva K, et al. Percentage low-density lipoprotein-cholesterol response to a given statin dose is not fixed across the pre-treatment range: Real world evidence from clinical practice: Data from the ESC-EORP EUROASPIRE V Study. Eur J Prev Cardiol. 2020;27(15):1630-6.

46. Zeller A, Taegtmeyer A, Martina B, Battegay E, Tschudi P. Physicians' ability to predict patients' adherence to antihypertensive medication in primary care. Hypertens Res. 2008;31(9):1765-71.

47. Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, et al. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. Eur Heart J. 2015;36(17):1012-22.

48. Wei MY, Ito MK, Cohen JD, Brinton EA, Jacobson TA. Predictors of statin adherence, switching, and discontinuation in the USAGE survey: understanding the use of statins in America and gaps in patient education. J Clin Lipidol. 2013;7(5):472-83.

49. Nieuwlaat R, Wilczynski N, Navarro T, Hobson N, Jeffery R, Keepanasseril A, et al. Interventions for enhancing medication adherence. Cochrane Database Syst Rev. 2014(11):CD000011.

50. Lennernäs H. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet. 2003;42(13):1141-60.

51. Vildhede A, Karlgren M, Svedberg EK, Wisniewski JR, Lai Y, Norén A, et al. Hepatic uptake of atorvastatin: influence of variability in transporter expression on uptake clearance and drug-drug interactions. Drug Metab Dispos. 2014;42(7):1210-8.

52. Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther. 2007;82(6):726-33.

53. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. Clin Pharmacol Ther. 2006;80(6):565-81.

54. Prueksaritanont T, Subramanian R, Fang X, Ma B, Qiu Y, Lin JH, et al. Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. Drug Metab Dispos. 2002;30(5):505-12.

55. Vethe NT, Munkhaugen J, Andersen AM, Husebye E, Bergan S. A Method for Direct Monitoring of Atorvastatin Adherence in Cardiovascular Disease Prevention: Quantification of the Total Exposure to Parent Drug and Major Metabolites Using 2-Channel Chromatography and Tandem Mass Spectrometry. Ther Drug Monit. 2019;41(1):19-28.

56. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. Lancet. 2010;375(9716):735-42.

57. Finegold JA, Manisty CH, Goldacre B, Barron AJ, Francis DP. What proportion of symptomatic side effects in patients taking statins are genuinely caused by the drug? Systematic review of randomized placebo-controlled trials to aid individual patient choice. Eur J Prev Cardiol. 2014;21(4):464-74.

58. Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol. 2006;97(8a):52c-60c.

59. Armitage J. The safety of statins in clinical practice. Lancet. 2007;370(9601):1781-90.

60. Ward NC, Watts GF, Eckel RH. Statin Toxicity. Circ Res. 2019;124(2):328-50.

61. Mosshammer D, Schaeffeler E, Schwab M, Morike K. Mechanisms and assessment of statin-related muscular adverse effects. Br J Clin Pharmacol. 2014;78(3):454-66.

62. Hermann M, Bogsrud MP, Molden E, Asberg A, Mohebi BU, Ose L, et al. Exposure of atorvastatin is unchanged but lactone and acid metabolites are increased several-fold in patients with atorvastatin-induced myopathy. Clin Pharmacol Ther. 2006;79(6):532-9.

63. Skottheim IB, Gedde-Dahl A, Hejazifar S, Hoel K, Asberg A. Statin induced myotoxicity: the lactone forms are more potent than the acid forms in human skeletal muscle cells in vitro. Eur J Pharm Sci. 2008;33(4-5):317-25.

64. Buettner C, Rippberger MJ, Smith JK, Leveille SG, Davis RB, Mittleman MA. Statin use and musculoskeletal pain among adults with and without arthritis. Am J Med. 2012;125(2):176-82.

65. Mansi I, Frei CR, Pugh MJ, Makris U, Mortensen EM. Statins and musculoskeletal conditions, arthropathies, and injuries. JAMA Intern Med. 2013;173(14):1-10.

66. Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. Cardiovasc Drugs Ther. 2005;19(6):403-14.

67. Cohen JD, Brinton EA, Ito MK, Jacobson TA. Understanding Statin Use in America and Gaps in Patient Education (USAGE): an internet-based survey of 10,138 current and former statin users. J Clin Lipidol. 2012;6(3):208-15.

68. Gupta A, Thompson D, Whitehouse A, Collier T, Dahlof B, Poulter N, et al. Adverse events associated with unblinded, but not with blinded, statin therapy in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid-Lowering Arm (ASCOT-LLA): a randomised double-blind placebo-controlled trial and its non-randomised non-blind extension phase. Lancet. 2017;389(10088):2473-81.

69. Nielsen SF, Nordestgaard BG. Negative statin-related news stories decrease statin persistence and increase myocardial infarction and cardiovascular mortality: a nationwide prospective cohort study. Eur Heart J. 2016;37(11):908-16.

70. Joy TR, Monjed A, Zou GY, Hegele RA, McDonald CG, Mahon JL. N-of-1 (single-patient) trials for statin-related myalgia. Ann Intern Med. 2014;160(5):301-10.

71. Rosenson RS, Baker SK, Jacobson TA, Kopecky SL, Parker BA, The National Lipid Association's Muscle Safety Expert P. An assessment by the Statin Muscle Safety Task Force: 2014 update. J Clin Lipidol. 2014;8(3 Suppl):S58-71.

72. Banach M, Rizzo M, Toth PP, Farnier M, Davidson MH, Al-Rasadi K, et al. Statin intolerance - an attempt at a unified definition. Position paper from an International Lipid Expert Panel. Arch Med Sci. 2015;11(1):1-23.

73. Statistikkbanken [Web page]. www.ssb.no/statbank: Statistics norway; 2021 [Available from: www.ssb.no/statbank.

74. Bioanalytical method validation [Web page]. www.ema.europa.eu: Eurpean Medicines Agency; 2020 [cited 2020 November 22nd]. Available from: https://www.ema.europa.eu/en/bioanalytical-method-validation.

75. Pallesen S, Bjorvatn B, Nordhus IH, Sivertsen B, Hjørnevik M, Morin CM. A new scale for measuring insomnia: the Bergen Insomnia Scale. Percept Mot Skills. 2008;107(3):691-706.

76. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373-83.

77. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the

diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal. 2016;37(27):2129-200.

78. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70.

79. Stone NJ, Robinson JG, Lichtenstein AH, Merz CNB, Blum CB, Eckel RH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. Circulation. 2014;129(25_suppl_2):S1-S45.

80. Denollet J. DS14: standard assessment of negative affectivity, social inhibition, and Type D personality. Psychosom Med. 2005;67(1):89-97.

81. Alpert BS. Validation of the Welch Allyn ProBP 3400: a device for modern medical practice. Blood Press Monit. 2011;16(3):156-8.

82. Stern RH, Yang BB, Hounslow NJ, MacMahon M, Abel RB, Olson SC. Pharmacodynamics and pharmacokinetic-pharmacodynamic relationships of atorvastatin, an HMG-CoA reductase inhibitor. J Clin Pharmacol. 2000;40(6):616-23.

83. Munkhaugen J, Sverre E, Peersen K, Gjertsen E, Gullestad L, Moum T, et al. The role of medical and psychosocial factors for unfavourable coronary risk factor control. Scand Cardiovasc J. 2016;50(1):1-8.

84. Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive validity of a medication adherence measure in an outpatient setting. J Clin Hypertens (Greenwich). 2008;10(5):348-54.

85. Gehi A, Haas D, Pipkin S, Whooley MA. Depression and medication adherence in outpatients with coronary heart disease: findings from the Heart and Soul Study. Arch Intern Med. 2005;165(21):2508-13.

86. Gehi AK, Ali S, Na B, Whooley MA. Self-reported medication adherence and cardiovascular events in patients with stable coronary heart disease: the heart and soul study. Arch Intern Med. 2007;167(16):1798-803.

87. Lowry R. VassarStats: Website for statistical computation www.vassarstats.net2022 [Available from: http://vassarstats.net/.

88. Munkhaugen J, Vethe NT, Fagerland MW, Dammen T, Perk J, Gjertsen E, et al. Statin-associated muscle symptoms in coronary patients: design of a randomized study. Scand Cardiovasc J. 2019;53(3):162-8.

89. Gallagher EJ, Liebman M, Bijur PE. Prospective validation of clinically important changes in pain severity measured on a visual analog scale. Ann Emerg Med. 2001;38(6):633-8.

90. Munkhaugen J. MUscle Side-Effects of Atorvastatin in Coronary Patients (MUSE) www.clinicaltrials.gov: U.S. National Library of Medicine; 2019 [cited 2022 March 17th]. Available from: https://clinicaltrials.gov/ct2/show/NCT03874156?term=munkhaugen&draw=2&rank=2.

91. Rezk MR, Badr KA. Quantification of amlodipine and atorvastatin in human plasma by UPLC-MS/MS method and its application to a bioequivalence study. Biomed Chromatogr. 2018;32(7):e4224.

92. Zhou Y, Li J, He X, Jia M, Liu M, Li H, et al. Development and validation of a liquid chromatography-tandem mass spectrometry method for simultaneous determination of amlodipine, atorvastatin and its metabolites ortho-hydroxy atorvastatin and para-hydroxy atorvastatin in human plasma and its application in a bioequivalence study. J Pharm Biomed Anal. 2013;83:101-7.

93. Turner RM, Fontana V, Bayliss M, Whalley S, Santoyo Castelazo A, Pirmohamed M. Development, validation and application of a novel HPLC-MS/MS method for the quantification of atorvastatin, bisoprolol and clopidogrel in a large cardiovascular patient cohort. J Pharm Biomed Anal. 2018;159:272-81.

94. Ryan TP, Morrison RD, Sutherland JJ, Milne SB, Ryan KA, Daniels JS, et al. Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring. PLoS One. 2017;12(9):e0185471.

95. Dormuth CR, Patrick AR, Shrank WH, Wright JM, Glynn RJ, Sutherland J, et al. Statin adherence and risk of accidents: a cautionary tale. Circulation. 2009;119(15):2051-7.

96. Simpson SH, Eurich DT, Majumdar SR, Padwal RS, Tsuyuki RT, Varney J, et al. A meta-analysis of the association between adherence to drug therapy and mortality. Bmj. 2006;333(7557):15.

97. Cheng CW, Woo KS, Chan JC, Tomlinson B, You JH. Assessing adherence to statin therapy using patient report, pill count, and an electronic monitoring device. Am J Health Syst Pharm. 2005;62(4):411-5.

98. Pedersen E, Truong KNL, Garcia BH, Halvorsen KH, Svendsen K, Eggen AE, et al. Self-reported medication use among coronary heart disease patients showed high validity compared with dispensing data. J Clin Epidemiol. 2021;135:115-24.

99. Gallagher BD, Muntner P, Moise N, Lin JJ, Kronish IM. Are two commonly used self-report questionnaires useful for identifying antihypertensive medication nonadherence? J Hypertens. 2015;33(5):1108-13.

100. Bergland OU, Halvorsen LV, Søraas CL, Hjørnholm U, Kjær VN, Rognstad S, et al. Detection of Nonadherence to Antihypertensive Treatment by Measurements of Serum Drug Concentrations. Hypertension. 2021;78(3):617-28.

101. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). European Heart Journal. 2018;39(33):3021-104.

102. Doshi JA, Zhu J, Lee BY, Kimmel SE, Volpp KG. Impact of a prescription copayment increase on lipid-lowering medication adherence in veterans. Circulation. 2009;119(3):390-7.

103. Felleskatalogen. Lipitor www.felleskatalogen.no: Felleskatalogen AS; 2022 [cited 2022 March 27th]. Available from: https://www.felleskatalogen.no/medisin/lipitor-upjohn-eesv-pfizer-560999.

104. Kronish IM, Rieckmann N, Halm EA, Shimbo D, Vorchheimer D, Haas DC, et al. Persistent depression affects adherence to secondary prevention behaviors after acute coronary syndromes. J Gen Intern Med. 2006;21(11):1178-83.

105. Williams L, O'Connor RC, Grubb N, O'Carroll R. Type D personality predicts poor medication adherence in myocardial infarction patients. Psychol Health. 2011;26(6):703-12.

106. Huber D, Wiken C, Henriksson R, Soderstrom L, Moore T. Statin treatment after acute coronary syndrome: Adherence and reasons for non-adherence in a randomized controlled intervention trial. Sci Rep. 2019;9(1):12079.

107. Bytyçi I, Penson PE, Mikhailidis DP, Wong ND, Hernandez AV, Sahebkar A, et al. Prevalence of statin intolerance: a meta-analysis. European Heart Journal. 2022.

108. Sverre E, Peersen K, Kristiansen O, Fagerland MW, Perk J, Husebye E, et al. Tailored clinical management after blinded statin challenge improved the lipid control in coronary patients with self-perceived muscle side effects. J Intern Med. 2022.

109. Herrett E, Williamson E, Brack K, Beaumont D, Perkins A, Thayne A, et al. Statin treatment and muscle symptoms: series of randomised, placebo controlled n-of-1 trials. Bmj. 2021;372:n135.

110. Wood FA, Howard JP, Finegold JA, Nowbar AN, Thompson DM, Arnold AD, et al. N-of-1 Trial of a Statin, Placebo, or No Treatment to Assess Side Effects. N Engl J Med. 2020;383(22):2182-4.

111. Howard JP, Wood FA, Finegold JA, Nowbar AN, Thompson DM, Arnold AD, et al. Side Effect Patterns in a Crossover Trial of Statin, Placebo, and No Treatment. J Am Coll Cardiol. 2021;78(12):1210-22.

112. Moriarty PM, Thompson PD, Cannon CP, Guyton JR, Bergeron J, Zieve FJ, et al. Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: The ODYSSEY ALTERNATIVE randomized trial. J Clin Lipidol. 2015;9(6):758-69.

113. Tinnermann A, Geuter S, Sprenger C, Finsterbusch J, Büchel C. Interactions between brain and spinal cord mediate value effects in nocebo hyperalgesia. Science. 2017;358(6359):105-8.

114. Skottheim IB, Bogsrud MP, Hermann M, Retterstol K, Asberg A. Atorvastatin metabolite measurements as a diagnostic tool for statin-induced myopathy. Mol Diagn Ther. 2011;15(4):221-7.

115. Cannon CP. Statin intolerance: how common is it and how do we work with patients to overcome it? European Heart Journal. 2022.

116. Sigglekow F, Horsburgh S, Parkin L. Statin adherence is lower in primary than secondary prevention: A national follow-up study of new users. PLoS One. 2020;15(11):e0242424.

117. Bjornsson TD, Wagner JA, Donahue SR, Harper D, Karim A, Khouri MS, et al. A Review and Assessment of Potential Sources of Ethnic Differences in Drug Responsiveness. The Journal of Clinical Pharmacology. 2003;43(9):943-67.

118. DeGorter MK, Tirona RG, Schwarz UI, Choi YH, Dresser GK, Suskin N, et al. Clinical and pharmacogenetic predictors of circulating atorvastatin and rosuvastatin concentrations in routine clinical care. Circ Cardiovasc Genet. 2013;6(4):400-8.

119. Peersen K, Munkhaugen J, Gullestad L, Dammen T, Moum T, Otterstad JE. Reproducibility of an extensive self-report questionnaire used in secondary coronary prevention. Scand J Public Health. 2017;45(3):269-76.

120. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248-52.

121. McGowan MP, Treating to New Target Study G. There is no evidence for an increase in acute coronary syndromes after short-term abrupt discontinuation of statins in stable cardiac patients. Circulation. 2004;110(16):2333-5.

122. Heeschen C, Hamm CW, Laufs U, Böhm M, Snapinn S, White HD. Withdrawal of Statins in Patients With Acute Coronary Syndromes. Circulation. 2003;107(3):e27-e.

123. Robinson JG. New insights into managing symptoms during statin therapy. Prog Cardiovasc Dis. 2019;62(5):390-4.

124. Peersen K, Munkhaugen J, Sverre E, Kristiansen O, Fagerland M, Vethe NT, et al. Clinical and psychological factors in coronary heart disease patients with statin associated muscle side-effects. BMC Cardiovasc Disord. 2021;21(1):596.

PAPER 1:

Oscar Kristiansen, Nils Tore Vethe,
Morten Wang Fagerland, Stein Bergan,
John Munkhaugen, Einar Husebye

A novel direct method to determine adherence to atorvastatin therapy in patients with coronary heart disease

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ORIGINAL ARTICLE

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A novel direct method to determine adherence to atorvastatin therapy in patients with coronary heart disease

Oscar Kristiansen[1,2†] | Nils T. Vethe[3†] | Morten W. Fagerland[4] | Stein Bergan[3,5] | John Munkhaugen[1,2] | Einar Husebye[1]

[1] Department of Medicine, Vestre Viken Trust, Drammen Hospital, Drammen, Norway

[2] Department of Behavioural Sciences in Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

[3] Department of Pharmacology, Oslo University Hospital, Oslo, Norway

[4] Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway

[5] School of Pharmacy, University of Oslo, Oslo, Norway

Correspondence
Oscar Kristiansen, Drammen Hospital, Vestre Viken HF, Postbox 800, 3004. Drammen, Norway.
Email: osckri@vestreviken.no

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Aims: Objective methods to monitor statin adherence are needed. We have established a liquid chromatography–tandem mass spectrometry assay for quantification of atorvastatin and its metabolites in blood. This study aimed to develop an objective drug exposure variable with cut-off values to discriminate among adherence, partial adherence and nonadherence to atorvastatin therapy in patients with coronary heart disease.

Methods: Twenty-five patients treated with atorvastatin 10 mg (n = 5), 20 mg (n = 6), 40 mg (n = 7) and 80 mg (n = 7) participated in a directly observed atorvastatin therapy study to confirm baseline adherence. After the directly observed therapy, half of the patients (*test group*) were instructed to stop taking atorvastatin and return for blood sample collection the subsequent 3 days. Levels of atorvastatin and metabolites were compared between the test group and the adherent control group.

Results: The sum of parent drug and all measured primary metabolites correlated well with the atorvastatin dose administered (Spearman's rho = 0.71, 95% CI 0.44–0.87). The dose-normalized atorvastatin plus metabolites concentrations completely separated the partially adherent test group from the controls at 0.18 nM/mg after 3 days without atorvastatin. To reduce the risk of misinterpreting adherent patients as partially adherent, a corresponding cut-off at 0.10 nM/mg is proposed. A metabolite level of 2-OH atorvastatin acid <0.014 nmol/L provided the optimal cut-off for nonadherence.

Conclusion: A direct method to discriminate among adherence, partial adherence and nonadherence to atorvastatin therapy in patients with coronary heart disease has been developed. This tool may be important for novel studies on adherence and potentially useful in clinical practice.

KEYWORDS

adherence, atorvastatin, coronary heart disease, liquid chromatography tandem mass spectrometry

PI Statement: The authors confirm that the Principal Investigator for this paper is John Munkhaugen and that he had direct clinical responsibility for patients.

[†]O. Kristiansen and N. T. Vethe contributed equally to this work

1 | INTRODUCTION

Poor adherence to statin treatment is a prevalent challenge in cardiovascular disease prevention,[1-3] associated with adverse outcomes.[4-6] Regular assessment of adherence is recommended by the European lipid guideline from 2016,[7] and was recently given a class IA recommendation in the corresponding US guideline.[8] Adherence has traditionally been monitored by indirect methods such as clinical judgement, self-reports or pill counts, methods that are prone to misinterpretation and overestimation of actual intake.[9] Prescription fill rates obtained from pharmacy registries provides the most comprehensive data on statin adherence today.[9,10] However, registry data is not feasible for documentation of statin intake in the individual patient. For statins, low-density lipoprotein cholesterol is an objective marker that might be used to monitor adherence. However, low-density lipoprotein cholesterol reduction secondary to statin therapy has been shown to vary from 5 to 70% between persons across all statin classes and doses.[11] Hence, objective methods to detect reduced adherence are requested[12] for the determination of true statin adherence in clinical practice.

Measurements of the active drug and/or its metabolites or directly observed therapy (DOT) are examples of objective methods for the assessment of drug adherence.[9] Assays for measuring statins and metabolites in blood with direct chromatography and tandem mass spectrometry (LC–MS/MS) methods have been described.[13-17] However, they are generally designed for studies on pharmacokinetics and bioequivalence.[13-17] We have recently reported a fast and reliable assay for the quantification of atorvastatin and its 5 major metabolites with LC–MS/MS methodology.[18] Importantly, this assay is feasible for the routine clinical laboratory with respect to technical implementation and interpretation of adherence.[18]

Atorvastatin is the most frequently used statin for the prevention of coronary heart disease (CHD) in Europe[19] and Norway.[20] Algorithms to allow discrimination among complete adherence, partial adherence and nonadherence to atorvastatin treatment, assessed by objective methods, have not yet been developed. Such algorithms may allow identification of patients at-risk of future treatment discontinuation and thus in need of closer follow-up. To be able to monitor adherence, a drug exposure variable with strong correlation to the ingested dose is required. Atorvastatin is converted to hydroxyl and lactone metabolites *in vivo*. CYP3A4 is primarily responsible for the hydroxylations[21] and the enzyme activity shows high variability between individuals.[22] Accordingly, the variability of atorvastatin metabolism needs to be levelled out in the context of a reliable drug exposure variable. The sum of atorvastatin and its major primary metabolites accounts for the major pharmacokinetic variability of this drug.[23]

We aimed to develop an objective drug exposure variable, reflecting the administered atorvastatin dose, with the ability to discriminate among adherence, partial adherence and nonadherence to atorvastatin therapy in CHD patients. We hypothesized, based on the reported half-life of atorvastatin in blood,[23] that adherence could be discriminated from partial adherence after the dose had been omitted for 1 to 3 days.

What is already known about this subject

- Poor statin adherence remains a prevalent challenge in patients with coronary heart disease associated with adverse outcomes.
- Objective methods, feasible for use in clinical practice and future research, allowing detection of poor atorvastatin adherence in spot blood samples, have previously not been described.

What this study adds

- Suggested dose-normalized cut-off values allowing discrimination among adherence and partial adherence to atorvastatin treatment in patients with coronary heart disease. A cut-off value to identify nonadherent patients (>3 daily doses omitted) is also proposed.
- The proposed method may be used to identify patients at-risk of future statin discontinuation and promote communication about adherence and side-effects. In turn, this may improve lipid management. The methodological approach may also translate to other drugs administered in chronic diseases.

2 | METHODS

2.1 | Design and patient characteristics

Twenty-five adult CHD patients treated with atorvastatin 10 mg (n = 5), 20 mg (n = 6), 40 mg (n = 7) and 80 mg (n = 7) were included in a clinical pharmacokinetic adherence study conducted from January to February 2018. Patients were recruited from the outpatient clinic at Drammen Hospital, Norway. A prerequisite for participation was no CHD events for 2 years prior to study participation and no present symptoms of unstable CHD. The exclusion criteria were medical or technical complications of blood sampling and difficulties collaborating with the study protocol. Patients were consecutively assigned to either the test or control group. One patient who did not comply with the study protocol was excluded, leaving 24 patients eligible for the analyses. A study flow chart is shown in Figure S1.

2.2 | Test procedure

Prior to inclusion, all patients participated in a 2-hour meeting with the study physicians where the background for the study and practical implementation was thoroughly explained. All patients were instructed to administer their atorvastatin dose between 7 and 10 AM once daily for at least 7 days prior to study start to ensure steady-state drug concentrations. On the first study day, all patients participated in a DOT study without having taken their morning dose. Blood samples were collected 1 hour before DOT (t_{-1}) and immediately before DOT (t_0) to

detect any unscheduled morning dose. Atorvastatin was then administered under observation by the study nurse and blood samples were collected 1 and 3 hours later in all patients. After the DOT study, half of the patients constituting the *control group* were not followed up further, whereas the *test group*, were instructed to stop taking atorvastatin and return for blood sampling after 24 (t_{24}), 48 (t_{48}), 72 (t_{72}) and 96 (t_{96}) hours. To provide data on drug and metabolite concentrations in patients with escape intake just prior to blood sampling, the test group also participated in a second DOT-study with blood sample collections 1 and 2 hours after atorvastatin administration (day 4, t_1 and t_2).

2.3 | Study assessments

2.3.1 | Assessment of atorvastatin and metabolites

Venous blood was sampled in EDTA vacutainers and handled according to both a low-temperature procedure and an ambient-temperature procedure as previously described.[18] The resulting plasma samples were analysed for the acid and lactone form of atorvastatin (parent drug), ortho- (2-OH) and para- (4-OH) hydroxyl atorvastatin with a 2-channel LC–MS/MS (Thermo Fisher Scientific, Waltham, MA, USA) at Oslo University Hospital. We have previously reported that the preanalytical stability of the acid forms are acceptable for 1 week at ambient and cool temperature, while the lactone forms demonstrate acceptable preanalytical stability for 3 hours at cool temperature (2–8°C).[18] Due to the in-sample conversions of the lactone forms to acids, we examined the sums of acid and corresponding lactone as this ensures stability of the drug concentration when samples are kept in ambient temperature for 6 days.[18]

Preset conditions for the alignment of optimal cut-off limits were: (i) to avoid that adherent patients are misclassified as partially adherent, a maximum of 1 day without drug intake was allowed; (ii) the cut-off between adherence and partial adherence aimed to indicate at least 2 days without drug intake; and (iii) the cut-off between partial and nonadherence aimed to indicate that >3 daily doses had been omitted. The sum of parent drug and the metabolites were evaluated as test variables to differentiate among adherence and partial adherence. Additionally, we assessed ratios between the parent drug and individual metabolites to compare the ability to discriminate partial adherence.

The lower limit of detection is set by a signal-to-noise ratio at 3 for the analyte. Since the concentration related to this limit will vary between samples, methods and laboratories it is unsuitable as a standardized cut-off limit for nonadherence. Therefore, a concentration threshold set at approximately 3-fold the lower limit of detection was defined for each substance (metabolite). The instrument response (i.e. analyte/internal standard ratio) corresponding to these standardized concentration-based thresholds were investigated as potential discriminators between partial and nonadherence. The individual half-lives were calculated for the parent drug and each metabolite in the test group, assuming first order kinetics and using linear regression of the Ln-transformed concentrations against time at t_{24}, t_{48}, and t_{72}. All test group patients were then simulated on each dose level, and the time to reach the lower concentration threshold was estimated for each substance, using the slope and intercept of the equations obtained by linear regression. The discriminating performance of the nonadherence limits will depend both on the pharmacokinetic characteristics and the analytical sensitivity of the method for the individual substance. Accordingly, the metabolite with superior ability to separate nonadherence from partial adherence could be identified.

In order to explain potential large deviations in drug or metabolite concentrations, we analysed relevant sequence variants in the *SLCO1B1*(c521T > C), *CYP3A4* (*22) and *CYP3A5* (*3) genes using real-time polymerase chain reaction amplification with hybridization probes and melt curve analysis (LightCycler 480, Roche Applied Science, Penzberg, Germany).

2.3.2 | Clinical data

Demographic and clinical covariates were obtained from hospital medical records, from a self-report questionnaire and a clinical examination completed at the first study visit. Study data included: age, sex, body weight and height, somatic comorbidities, food intake prior to drug administration, perceived statin-associated muscle symptoms, concurrent medication, and intake of grapefruit, St John's wort and red-yeast rice. Routine analysis of creatine kinase, creatinine, lactate dehydrogenase, alanine transaminase, aspartate transaminase, c-reactive protein and albumin were performed on a clinical chemistry analyser (Architect ci16200, Abbot Laboratories, Abbot Park, IL, USA).

2.4 | Ethics and safety

The study was conducted in accordance to the ethical principles of the Declaration of Helsinki and in consistence with ICH/Good Clinical Practice. The study protocol was reviewed by the Regional Committee for Medical and Health Research Ethics without remarks and approved by the local Data Protection Officer (16/00117–107). The Norwegian Medicines Agency did not define the study as a clinical trial requiring approval since the main purpose was not to ascertain or verify/compare the efficacy or safety of atorvastatin. All patients gave a written informed consent to participate prior to study start.

2.5 | Statistical analysis

Statistical analyses were performed using SPSS version 25. The Student *t* test was applied to assess differences in means (standard deviation) between the control and test group for continuous variables. The Fisher mid-*P* test was used for categorical variables.[24] Atorvastatin pharmacokinetic variables were assessed with the Mann–Whitney *U* test for unrelated samples, and the Wilcoxon signed rank test for paired samples. Correlations of parent drug and metabolites *vs* atorvastatin dose were assessed using Spearman's rank correlation. Linear regression analyses were applied to explore the associations between clinical and demographic variables and dose-adjusted atorvastatin and metabolite concentrations. Cut-off values for partial nonadherence were obtained with receiver operating characteristics curves at the different time intervals without dosing in the test group as compared to the completely adherent control group.

3 | RESULTS

Adherence at baseline was demonstrated with no significant differences in dose-normalized concentrations of atorvastatin plus metabolites immediately before DOT and 24 hours after DOT (median 0.46 *vs* 0.47 [nmol/L]/mg, *P* = .39). The demographic and clinical characteristics were comparable between the test group and the control group (Table 1).

Atorvastatin acid (parent drug) and its corresponding lactone constituted on average 37% (range 15–60%) of the sum of parent drug and all metabolites, whereas the 2-OH and 4-OH metabolites amounted to 44% (range 27–67%) and 19% (range 10–31%), respectively. The following half-lives (median, range) were calculated in the test group: atorvastatin acid 14 (11–24) hours, atorvastatin lactone 13 (10–20) hours, 2-OH atorvastatin acid 15 (12–48) hours, 2-OH atorvastatin lactone 15 (11–37) hours, 4-OH atorvastatin acid 21 (14–42) hours and 4-OH atorvastatin lactone 19 (13–40) hours. The sum of parent drug and all metabolites in blood samples handled by the ambient temperature procedure was mean 94% (range 77–130%, 95% CI 89–99%) compared to samples handled at cold temperature at t_0 and mean 96% (range 69–150%, 95% CI 81–111%) at t_{96}.

All measurements of parent drug and metabolites were above the lower limit of quantification (LLOQ) when patients were adherent to dose before DOT and 24 hours after DOT. Fifteen percent of the measurements were below LLOQ when doses were omitted in the test group.

3.1 | The relationship between atorvastatin dose and exposure

Correlations between the different drug exposure variables and the atorvastatin dose are shown in Table 2. There was a positive correlation between all individual analytes and the dose. The parent drug was numerically weaker correlated than the parent drug plus metabolites exposure.

TABLE 1 Patient characteristics

Characteristic	Control group (*n* = 12)	Test group (*n* = 12)	*P*-value
Age (y), mean (SD)	65.9 (8.7)	66.3 (12.7)	.941
Male, *n* (%)	9 (75)	10 (83)	.660
Atorvastatin dose (mg), mean (SD)	38 (29)	44 (27)	.617
Body weight (kg), mean (SD)	90.3 (11.0)	88.8 (15.9)	.802
ALT (U/L), mean (SD)	29.9 (18.0)	34.9 (17.3)	.495
CK (U/L), mean (SD)	102 (36)	145 (173)	.418
Albumin (g/L), mean (SD)	38.5 (4.8)	37.8 (2.5)	.636
LDH (mmol/L), mean (SD)	256 (38.4)	201 (41)	.154
LDL-C (mmol/L), mean (SD)	2.2 (0.7)	2.2 (0.4)	.815
Number of concomitant medications, mean (SD)	5.3 (1.8)	5.2 (2.4)	.925

SD; standard deviation, ALT; alanine aminotransferase, CK; creatinine kinase, LDH; lactate dehydrogenase, LDL-C; low density lipoprotein cholesterol

TABLE 2 Correlation of drug derivatives with atorvastatin dose

Analyte	Spearman's rho	95% CI	*P*-value
ATV acid (parent drug)	0.598	0.257–0.806	.002
ATV acid and lactone	0.587	0.241–0.800	.003
2OH ATV acid and lactone	0.752	0.501–0.886	<.001
4OH ATV acid and lactone	0.713	0.435–0.867	<.001
ATV acid +2OH ATV acid and lactone	0.697	0.409–0-858	<.001
ATV acid +4OH ATV acid and lactone	0.626	0.298–0.821	.001
ATV acid + all 5 metabolites	0.714	0.437–0.867	<.001

Obtained with Spearman's correlation using t_0 samples handled according to the low temperature procedure. ATV, atorvastatin; 2OH, ortho-hydroxyl; 4OH, para-hydroxyl

3.2 | Factors associated with the drug exposure

Increasing age was associated with increasing dose-normalized trough concentrations of atorvastatin acid (t_0; β from linear regression = 0.002 [95% CI 0.000–0.003], *P* = .019), atorvastatin acid plus lactone (β = 0.004 [95% CI 0.000–0.008], *P* = .044) and atorvastatin acid plus all metabolites (β = 0.010 [95% CI 0.002–0.017], *P* = .012). Sex, body weight, renal function, food intake prior to drug administration and perceived statin side effects were not associated with significant variations in atorvastatin and metabolites concentrations at t_0.

Compared with patients expressing the wild types, the mean dose-normalized t_0 concentration sum of atorvastatin acid plus all metabolites were not statistically different in patients expressing variants in *SLCO1B1* (*n* = 7), *CYP3A4* (*n* = 2) or *CYP3A5* (*n* = 3): 0.36 *vs* 0.49 nM/mg, 0.55 *vs* 0.44 nM/mg, 0.43 *vs* 0.46 nM/mg, respectively.

3.3 | Identification of patients with partial adherence

When the test group had omitted tablet intake for 3 days, all the individual dose-normalized sums of atorvastatin plus 5 metabolites (6-component sum) were separated from the corresponding sums in the controls, i.e. discriminated at 0.20 nM/mg for samples handled at low temperature and at 0.18 nM/mg for samples handled at ambient temperature (Figure 1). For this 6-component sum, the receiver operating characteristics curve analysis with cut-off at 0.10 nM/mg provided 100% sensitivity and 92% specificity for the identification of partial adherence when 2 or 3 doses were omitted, regardless of the preanalytical sample handling procedure (Table 3).

The dose-normalized sum of the acid and lactone form of the parent drug (2-component sum) was also assessed, and the respective groups where entirely separated at 0.070 nM/mg after the test group had omitted 2 doses (Figure 2). A cut-off at 0.050 nM/mg thus provided 100% sensitivity and 100% specificity for the identification of

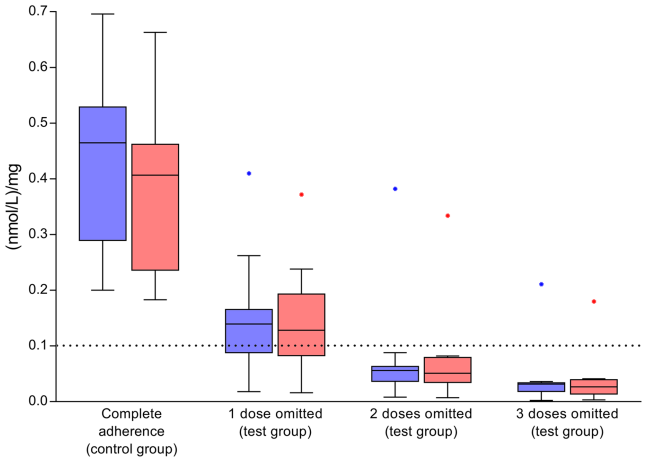

FIGURE 1 Tukey box-and-whisker plots of dose-normalized concentration sums of atorvastatin acid and 5 metabolites (nM/mg) in the completely adherent control group and the test group omitting drug intake over a period of 3 days. Note: dotted line represents the suggested cut-off for partial nonadherence at 0.10 nM/mg. Blue and red boxes show samples handled in low and ambient temperature, respectively. Dots represent a single outlier patient

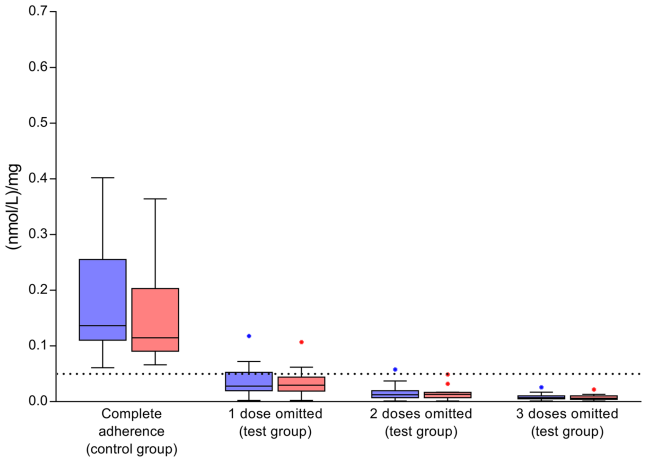

FIGURE 2 Tukey box-and-whisker plots of dose-normalized concentration sums of atorvastatin acid and lactone (nM/mg) in the completely adherent control group and the test group omitting drug intake over a period of 3 days. Note: dotted line represents the suggested cut-off for partial nonadherence at 0.05 nM/mg. Blue and red boxes show samples handled in low and ambient temperature, respectively. Dots represent a single outlier patient

partial adherence when 2 or 3 doses were omitted, regardless of sample handling procedure (Table 3). Ratios between the acid plus lactone form of atorvastatin and 2-OH atorvastatin, as well as ratios between the acid plus lactone form of atorvastatin and 4-OH atorvastatin, discriminated less adequately between adherence and partial adherence (data not shown).

3.4 | Identification of nonadherence

The metabolite 2-OH atorvastatin acid (1-component), with instrument response corresponding to a standardized concentration threshold at 0.014 nmol/L, showed superior ability to distinguish nonadherence (omitting dose for >3 days) from partial adherence. The time to reach this threshold, after cessation of drug intake, was median 3.8 (range 2.2–14) days at 10 mg, 4.3 (2.8–16) days at 20 mg, 5.0 (3.3–18) days at 40 mg, and 5.6 (3.8–20) days at 80 mg. These pharmacokinetic simulations were based on linear regression with R^2 at median 0.988 (0.866–1.000). With 2-OH atorvastatin acid below 0.014 nmol/L, 100% were correctly classified as nonadherent at the 40- and 80-mg dose levels, and 83% at the 10- and 20-mg dose levels (17% misclassified as nonadherent when being partially adherent).

TABLE 3 Sensitivity and specificity of suggested cut-off values to discriminate partial from complete adherence

Analyte	Discriminator nM/mg	1 dose omitted	2 doses omitted	3 doses omitted
ATV acid and lactone (low)	0.05	100/75	100/92	100/100
ATV acid and lactone (ambient)	0.05	100/83	100/100	100/100
ATV plus all metabolites (low)	0.10	100/42	100/92	100/92
ATV plus all metabolites (ambient)	0.10	100/42	100/92	100/92

Results from receiver operating characteristics curve analysis presented as sensitivity (%) /specificity (%). The discriminator represents the dose-normalized sum of concentrations. Low and ambient refer to the temperature during the preanalytical sample handling. ATV, atorvastatin

3.5 | Identification of an unscheduled dose prior to blood sampling

The maximum dose-normalized 2-component sum at t_0 in the pooled control and test group was 0.40 (cold temperature) and 0.36 (ambient temperature) nM/mg. The corresponding mean concentration after the second DOT study was 1.74 (range 0.03–5.61) nM/mg at day 4, t_1 and 2.21 (range 0.09–5.64) nM/mg at day 4, t_2. At day 4, t_1 and t_2, respectively, 33% and 17% of the test group were below 0.40 nM/mg.

4 | DISCUSSION

To our knowledge, this is the first study to present a test procedure with cut-off values that allow discrimination among adherence, partial adherence and nonadherence to atorvastatin therapy, based on LC–MS/MS measurements of plasma drug concentrations. By the present analysis and algorithm, patients at risk for permanent statin discontinuation may be evaluated directly, by a single blood test at expected steady state, with regard to statin adherence.

There is no consensus with regards to the definition of adherence, partial adherence and nonadherence in the statin literature.[7,9,12] Indeed, this also applies for objective methods to monitor other cardiovascular drugs. Accordingly, the present methodological approach

may also translate to determine the adherence to other drugs administered in chronic diseases. To our knowledge, only 1 previous study has applied an LC–MS/MS assay in clinical blood samples to determine adherence to atorvastatin.[25] A dichotomous classification of adherence based on atorvastatin blood concentration over or under the LLOQ was used.[25]

Our adherence algorithm classifies adherence, partial adherence and nonadherence. With the chosen drug exposure variable and cut-off, partial adherence implies that the dose is omitted for up to 3 days. A 6-component sum (dose-normalized atorvastatin acid plus all metabolites) <0.10 nM/mg provides 100% sensitivity and 92% specificity when 2 or 3 doses are omitted. Forty-two percent will be classified with partial adherence if a single dose is omitted (Figure 1). This cut-off was selected as a practical approach to minimize the risk of classifying adherent patients as partially adherent and to reduce the effect of a single apparent outlier in the data set. Standardized conditions for blood sampling are important to ensure the given diagnostic sensitivity and specificity. Thus, we recommend blood sampling just prior to the next scheduled dose, i.e. trough concentration.

We also present an alternative approach to classify partial adherence, by using a 2-component sum (dose-normalized atorvastatin acid plus lactone). A cut-off at <0.05 nM/mg provides 100% sensitivity and 100% specificity when 2 or 3 doses are omitted, due to the faster elimination of these substances. However, more patients (i.e. 75–83%) will be classified with partial adherence by the 2-component sum when a single dose is omitted (Figure 2). The significance of omitting a single dose only for the evaluation of adherence is debatable, and partial adherence was intended to indicate 2–3 days without drug intake. Partial adherence will thus be slightly more severe and optimally classified by the 6-component model, according to the aims for the method. This gain needs to be balanced towards the simpler and less expensive 2-component model. The correlation to the given dose was higher for the 6-component sum, and the 2-component model may be more prone to variations in drug metabolism. A similar effect can be assumed for interacting drugs, not examined in the present study. Further studies will confirm the optimal method to distinguish adherence and partial adherence.

The categorization of nonadherence solely on the basis of nondetectable *vs* detectable drug levels in blood has important limitations with respect to standardization. In general, the lower limit of detection is below the quantitative range, and it is dependent on factors displaying variability within and between laboratories (i.e. methodological conditions, sample composition and LC–MS/MS instrument sensitivity). It would not be possible to standardize terms such as nonadherence, poor adherence or low adherence with respect to time intervals without dosing, if a nondetectable drug level is the only criterion for classification. Our concept allows nonadherence to be defined in terms of a minimum time interval omitting dosing. Thus, we suggest a standardized lower concentration-based threshold as cut-off to identify nonadherent patients. Since this concentration is below the LLOQ, our practical approach is to apply the instrument response (analyte/internal standard ratio) that corresponds to this lower concentration threshold. Although measurements above the LLOQ would be optimal for any purpose, the semiquantitative approach allows interpretations in relation to days without dosing, and the internal standard adjustment allows correction for matrix effects between samples and other methodological fluctuations. The specific nonadherence threshold was elucidated with samples handled according to the low-temperature procedure, and 2-OH atorvastatin acid <0.014 nmol/L demonstrated to be the optimal discriminator for nonadherence (corresponding to >3 consecutive days without dosing). Nevertheless, this 1-component model should also be applicable when the ambient temperature sample handling procedure is applied, although the misclassification as partially adherent then may be increased due to the preanalytical lactone-to-acid conversion. Due to the semiquantitative nature of the nonadherence cut-off, its accuracy was estimated with the proportion of correctly classified patients (100% at 40 and 80 mg, and 83% at 10 and 20 mg). The clinical relevance of the nonadherence cut-off, and also the relevance of differentiation between partial adherence and nonadherence, should be validated in a larger clinical study.

The preanalytical procedures are simplified by using the sum of acid and lactone forms, allowing blood samples to be handled in ambient temperature.[18] In the present study, we demonstrate that the proposed cut-offs are equal for samples handled at ambient temperature, a major advantage for the potential use of the test in routine clinical practice. The 6-component sum correlated well with the administered dose at steady state, suggesting this would be a representative drug exposure variable with the benefit of levelling out within- and between-individual variations in drug metabolism.[22]

Increasing age was associated with higher dose-normalized blood concentrations of both parent drug and metabolites, a finding supported by previous studies examining factors associated with variations in atorvastatin concentrations.[26,27] Due to the limited sample size, we did not attempt to age adjust the drug exposure variable, but this should be considered in a larger cohort.

The recent emergence of new, expensive drugs targeting subclinical inflammation[28] and lowering lipids,[29] emphasizes the need to improve adherence to the cost-effective statins. Knowledge about the prevalence of execution issues (i.e. omitting doses) was strongly requested in a recent position paper from the European Society of Cardiology.[30] Our new direct method combined with the algorithms developed in the present study can be used in future studies to differentiate and describe the prevalence of adherence, partial adherence and nonadherence to atorvastatin therapy. When combined with clinical data, causes of partial and nonadherence to atorvastatin therapy may be revealed to develop new approaches for improving adherence. Measurement of plasma drug levels revealed that nonadherence was common in patients with apparent treatment resistant hypertension in 2 recent studies.[31,32] Data from a pilot study in patients with low or undetectable blood levels of blood pressure-lowering drugs indicated that confronting these patients with the study results, improved adherence and reduced average blood pressure with >15 mmHg.[33] Thus, a test for atorvastatin adherence may enhance the clinicians' awareness at follow-up visits and encourage communication about adherence between physician and patient.

4.1 | Limitations

Due to the limited sample size there is a risk of bias and spurious results when multiple-adjusted analyses are performed. Hence, patient factors besides age may be associated with variations in drug or metabolites exposure. The suggested cut-off values should therefore be validated, and potentially adjusted, in a larger data set including more patients with multiple comorbidities, interacting drugs and genetic variations that may influence the statin pharmacokinetics. The present methodology should also be cross-validated with other adherence assessment methods. Patients with extremely high or low atorvastatin or metabolite concentrations could potentially be misclassified with the suggested algorithms. Further knowledge may guide the interpretation of the test when risk factors for misclassification are present. Even though we identified a cut-off that allows detection of escape dose intake 1 or 2 hours prior to blood sampling in a majority (i.e. 67 and 83%, respectively) of the partially adherent patients, the risk of white coat adherence is not eliminated.[9] Finally, concentrations below the LLOQ were used, although it brings along uncertainty. Achievement of a lower LLOQ should be addressed in future improvement of the methodology.

5 | CONCLUSION

Cut-off values based on the pharmacokinetics of atorvastatin and metabolites in spot blood samples, allowing discrimination among adherence, partial and nonadherence to atorvastatin therapy in CHD patients have been developed. The present direct method to determine atorvastatin adherence may optimize the use of cost-effective statins in clinical practice to improve lipid management and clinical outcomes.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

O.K., N.T.V. and J.M. drafted the manuscript. O.K., N.T.V., J.M., M.W.F., S.B. and E.H revised the manuscript. O.K., N.T.V., S.B., J.M., E.H and M. W.F analysed the data. N.T.V., J.M, S.B. and E.H designed the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Oscar Kristiansen https://orcid.org/0000-0002-4539-0239

REFERENCES

1. Benner JS, Glynn RJ, Mogun H, Neumann PJ, Weinstein MC, Avorn J. Long-term persistence in use of statin therapy in elderly patients. *JAMA*. 2002;288(4):455-461.
2. Hirsh BJ, Smilowitz NR, Rosenson RS, Fuster V, Sperling LS. Utilization of and adherence to guideline-recommended lipid-lowering therapy after acute coronary syndrome: opportunities for improvement. *J am Coll Cardiol*. 2015;66(2):184-192.
3. Ofori-Asenso R, Ilomaki J, Tacey M, et al. Predictors of first-year nonadherence and discontinuation of statins among older adults: a retrospective cohort study. *Br J Clin Pharmacol*. 2019;85(1):227-235.
4. Turner RM, Yin P, Hanson A, et al. Investigating the prevalence, predictors, and prognosis of suboptimal statin use early after a non-ST elevation acute coronary syndrome. *J Clin Lipidol*. 2017;11(1):204-214.
5. Sokol MC, McGuigan KA, Verbrugge RR, Epstein RS. Impact of medication adherence on hospitalization risk and healthcare cost. *Med Care*. 2005;43(6):521-530.
6. De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. *Br J Clin Pharmacol*. 2014;78(4):684-698.
7. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the Management of Dyslipidaemias: the task force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European atherosclerosis society (EAS) developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis*. 2016;253:281-344.
8. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the Management of Blood Cholesterol: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *J am Coll Cardiol* 2018; 139:1106.
9. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med*. 2005;353(5):487-497.
10. Ho PM, Bryson CL, Rumsfeld JS. Medication adherence: its importance in cardiovascular outcomes. *Circulation*. 2009;119(23):3028-3035.
11. Karlson BW, Wiklund O, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: results from VOYAGER. *Eur Heart J Cardiovasc Pharmacother*. 2016;2(4):212-217.
12. Nieuwlaat R, Wilczynski N, Navarro T, et al. Interventions for enhancing medication adherence. *Cochrane Database Syst Rev*. 2014;(11):CD000011.
13. Wang J, Luzum JA, Phelps MA, Kitzmiller JP. Liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of simvastatin, lovastatin, atorvastatin, and their major metabolites in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2015;983-984:18-25.
14. Zhou Y, Li J, He X, et al. Development and validation of a liquid chromatography-tandem mass spectrometry method for simultaneous determination of amlodipine, atorvastatin and its metabolites ortho-hydroxy atorvastatin and Para-hydroxy atorvastatin in human plasma and its application in a bioequivalence study. *J Pharm Biomed Anal*. 2013;83:101-107.

15. Vlckova H, Svoboda P, Novak O, Solich P, Novakova L. Development of MEPS-UHPLC-MS/MS multistatin methods for clinical analysis. *Bioanalysis*. 2016;8(4):333-349.
16. Yacoub M, Awwad AA, Alawi M, Arafat T. Simultaneous determination of amlodipine and atorvastatin with its metabolites; ortho and Para hydroxy atorvastatin; in human plasma by LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2013;917-918:36-47.
17. Rezk MR, Badr KA. Quantification of amlodipine and atorvastatin in human plasma by UPLC-MS/MS method and its application to a bio-equivalence study. *Biomed Chromatogr*. 2018;32(7):e4224.
18. Vethe NT, Munkhaugen J, Andersen AM, Husebye E, Bergan S. A method for direct monitoring of atorvastatin adherence in cardiovascular disease prevention: quantification of the Total exposure to parent drug and major metabolites using 2-channel chromatography and tandem mass spectrometry. *Ther Drug Monit*. 2019;41(1):19-28.
19. Alnouri F, Wood D, Kotseva K, Ibrahim ME. Which statin worked best to achieve lipid level targets in a European registry? A post-hoc analysis of the EUROASPIRE III for coronary heart disease patients. *J Saudi Heart Assoc*. 2014;26(4):183-191.
20. Munkhaugen J, Sverre E, Otterstad JE, et al. Medical and psychosocial factors and unfavourable low-density lipoprotein cholesterol control in coronary patients. *Eur J Prev Cardiol*. 2017;24(9):981-989.
21. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther*. 2006;80(6):565-581.
22. Hohmann N, Haefeli WE, Mikus G. CYP3A activity: towards dose adaptation to the individual. *Expert Opin Drug Metab Toxicol*. 2016;12(5):479-497.
23. Lennernas H. Clinical pharmacokinetics of atorvastatin. *Clin Pharmacokinet*. 2003;42(13):1141-1160.
24. Fagerland MW, Lydersen S, Laake P. *Statistical Analysis of Contingency Tables*. Boca Raton, FL: Chapman & Hall/CRC; 2017 Chapter 4.
25. Ryan TP, Morrison RD, Sutherland JJ, et al. Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring. *PLoS One*. 2017;12(9):e0185471.
26. Gibson DM, Bron NJ, Richens A, Hounslow NJ, Sedman AJ, Whitfield LR. Effect of age and gender on pharmacokinetics of atorvastatin in humans. *J Clin Pharmacol*. 1996;36(3):242-246.
27. DeGorter MK, Tirona RG, Schwarz UI, et al. Clinical and pharmacogenetic predictors of circulating atorvastatin and rosuvastatin concentrations in routine clinical care. *Circ Cardiovasc Genet*. 2013;6(4):400-408.
28. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with Canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377(12):1119-1131.
29. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. 2018;379(22):2097-2107.
30. Vonbank A, Agewall S, Kjeldsen KP, et al. Comprehensive efforts to increase adherence to statin therapy. *Eur Heart J*. 2017;38(32):2473-2479.
31. de Jager RL, van Maarseveen EM, Bots ML, Blankestijn PJ, SYMPATHY investigators. Medication adherence in patients with apparent resistant hypertension: findings from the SYMPATHY trial. *Br J Clin Pharmacol*. 2018;84(1):18-24.
32. Avataneo V, De Nicolo A, Rabbia F, et al. Therapeutic drug monitoring-guided definition of adherence profiles in resistant hypertension and identification of predictors of poor adherence. *Br J Clin Pharmacol*. 2018;84(11):2535-2543.
33. Brinker S, Pandey A, Ayers C, et al. Therapeutic drug monitoring facilitates blood pressure control in resistant hypertension. *J am Coll Cardiol*. 2014;63(8):834-835.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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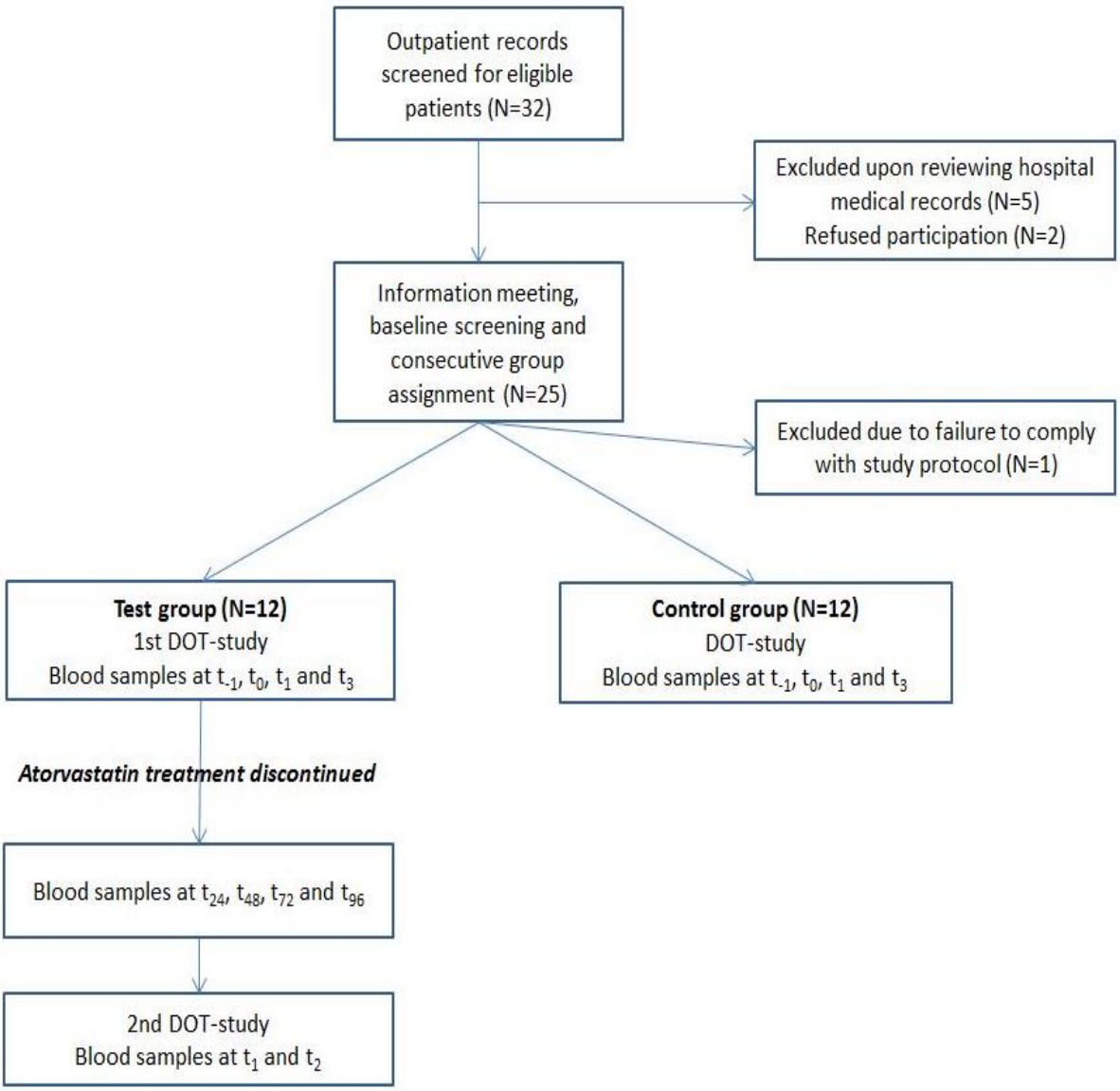
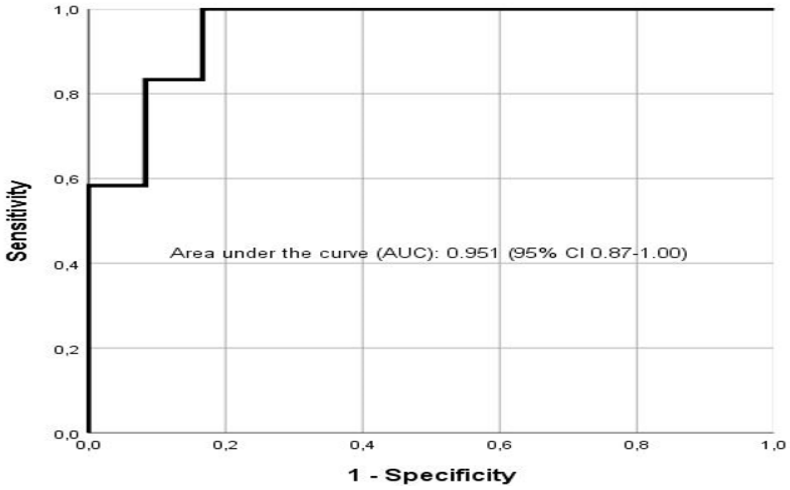
SUPPLEMENTARY MATERIAL PAPER 1

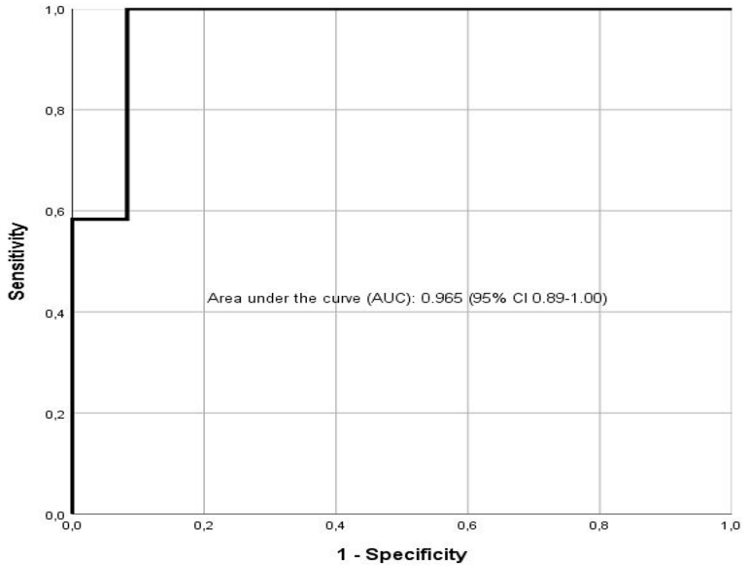
Paper 1 - Supplementary Figure 1 – Study flow chart



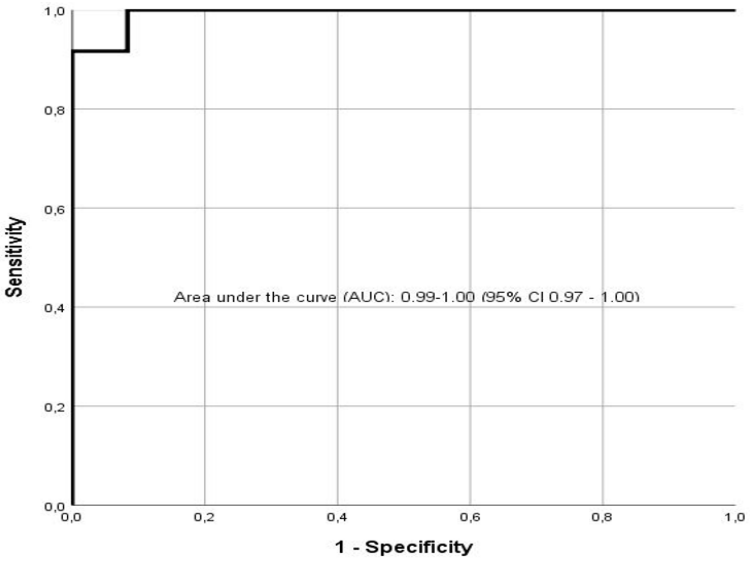
Paper 1 - Supplementary Figure 2 – ROC six-component sum 1 dose omitted



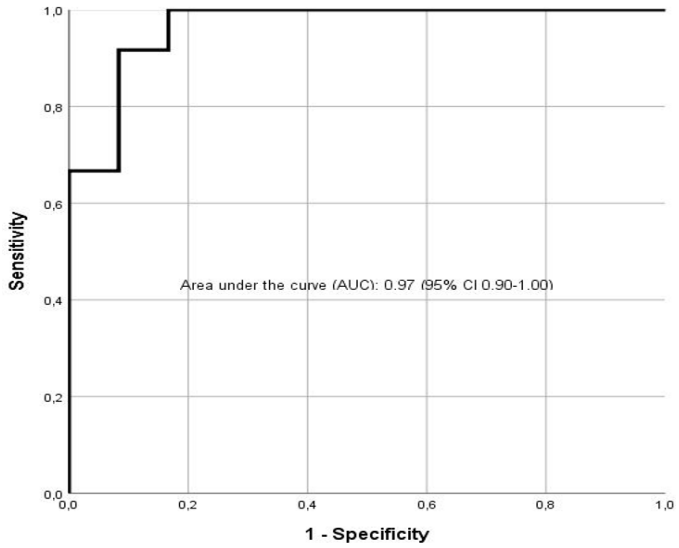
Paper 1 - Supplementary Figure 3 – ROC six-component sum 2 doses omitted



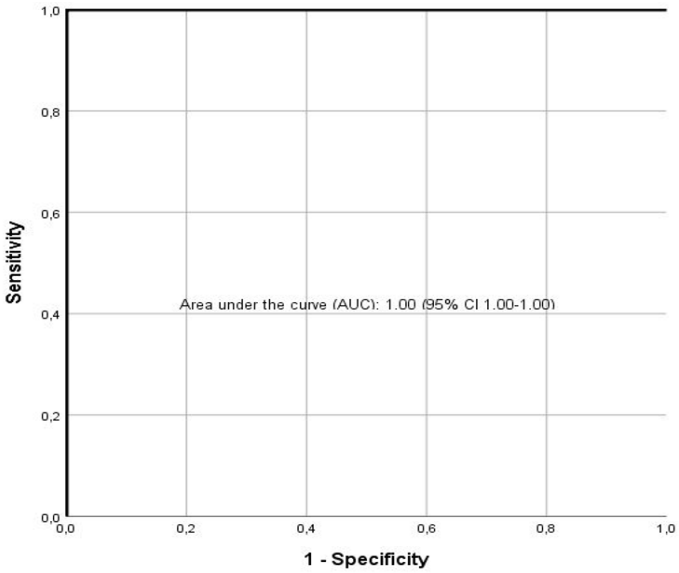
Paper 1 - Supplementary Figure 4 – ROC six-component sum 3 doses omitted



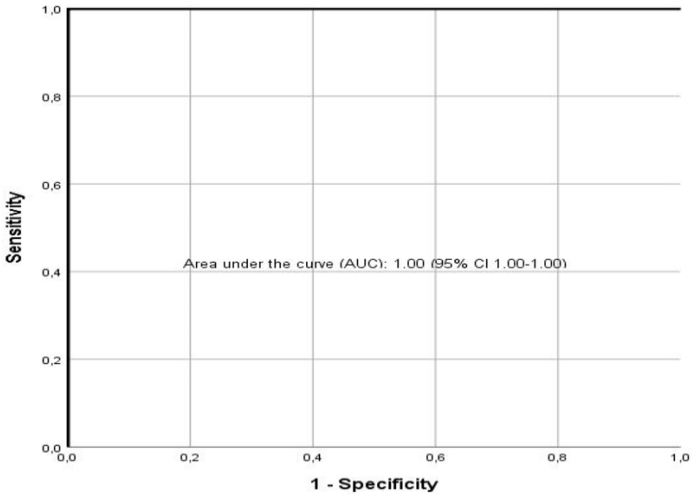
Paper 1 - Supplementary Figure 5 – ROC two-component sum 1 dose omitted



Paper 1 - Supplementary Figure 6 – ROC two-component sum 2 doses omitted



Paper 1 - Supplementary Figure 7 – ROC two-component sum 3 doses omitted



PAPER 2:

Oscar Kristiansen, Elise Sverre, Kari Peersen,
Morten Wang Fagerland, Erik Gjertsen, Lars Gullestad,
Joep Perk, Toril Dammen, Einar Husebye, Nils Tore Vethe,
John Munkhaugen

The relationship between directly measured statin adherence, self-reported adherence measures and cholesterol levels in patients with coronary heart disease

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The relationship between directly measured statin adherence, self-reported adherence measures and cholesterol levels in patients with coronary heart disease

Oscar Kristiansen [a,b,*], Elise Sverre [a,b], Kari Peersen [c], Morten Wang Fagerland [d], Erik Gjertsen [a], Lars Gullestad [e,f,g], Joep Perk [h], Toril Dammen [b], Einar Husebye [a], Nils Tore Vethe [i], John Munkhaugen [a,b]

[a] Department of Medicine, Drammen Hospital, Vestre Viken Trust, Dronninggata 28, 3004, Drammen, Norway
[b] Department of Behavioral Medicine, Faculty of Medicine, University of Oslo, Domus Medica, Sognsvannsveien 9, 0372, Oslo, Norway
[c] Department of Cardiology, Vestfold Hospital Trust, Halfdan Wilhelmsens alle 17, 3103, Tønsberg, Norway
[d] Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Domus Medica, Sognsvannsveien 9, 0372, Oslo, Norway
[e] Department of Cardiology, Oslo University Hospital Rikshospitalet, Sognsvannsveien 20, 0372, Oslo, Norway
[f] Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Sognsvannsveien 20, 0372, Oslo, Norway
[g] KG Jebsen Cardiac Research Centre, Oslo University Hospital, Sognsvannsveien 20, 0372, Oslo, Norway
[h] Department of Cardiology, Public Health Department, Linnaeus University, 391 82, Kalmar, Sweden
[i] Department of Pharmacology, Oslo University Hospital, Sognsvannsveien 20, 0372, Oslo, Norway

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ABSTRACT

Background and aims: We aimed to determine the relationship between statin adherence measured directly, and by self-report measures and serum cholesterol levels.
Methods: Patients prescribed atorvastatin (N = 373) participated in a cross-sectional study 2–36 months after a coronary event. Self-reported adherence included statin adherence the past week, the 8-item Morisky medication adherence scale (MMAS-8), and the Gehi et al. adherence question. Atorvastatin was measured directly in spot blood plasma by a novel liquid chromatography tandem mass-spectrometry method discriminating adherence (0–1 doses omitted) and reduced adherence (≥2 doses omitted). Participants were unaware of the atorvastatin analyses at study participation.
Results: Mean age was 63 (SD 9) years and 8% had reduced atorvastatin adherence according to the direct method. In patients classified with reduced adherence by the direct method, 40% reported reduced statin adherence, 32% reported reduced adherence with the MMAS-8 and 22% with the Gehi question. In those adherent by the direct method, 96% also reported high statin adherence, 95% reported high adherence on the MMAS-8 whereas 94% reported high adherence on the Gehi question. Cohen's kappa agreement score with the direct method was 0.4 for self-reported statin adherence, 0.3 for the Gehi question and 0.2 for the MMAS-8. Adherence determined by the direct method, self-reported statin adherence last week, and the Gehi question was inversely related to LDL-cholesterol levels with a *p*-value of <0.001, 0.001 and 0.004, respectively.
Conclusions: Plasma-statin measurements reveal reduced adherence with higher sensitivity than self-report measures, relate to cholesterol levels, and may prove to be a useful tool to improve lipid management.

1. Introduction

Despite extensive evidence proving the beneficial effect of statins on clinical outcomes with low rates of side-effects [1,2] poor adherence remains common [3]. Insufficient statin therapy is associated with an increased risk of major adverse cardiovascular events [4] and a graded, inverse association between statin adherence and mortality has been shown for patients with established cardiovascular disease (CVD) [5]. Accordingly, reduced adherence is a major barrier to address in order to achieve successful prevention of CVD [6]. Reliable methods that can be

* Corresponding author. Department of Medicine, Drammen Hospital, Vestre Viken Trust, Dronninggata 28, 3004, Drammen, Norway.
E-mail address: osckri@vestreviken.no (O. Kristiansen).

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used in clinical practice to identify the individual patients with low statin adherence are required for the implementation of evidence-based interventions that improve adherence [7] as recommended by recent European and US lipid guidelines [8,9].

Methods for assessing adherence to medication are often divided into indirect or direct methods [10]. Indirect methods include clinical judgement, self-report questionnaires, pill-counts, pharmacy registry data and electronic medication event monitoring systems. In general, all indirect methods are limited in the sense that they do not document actual intake of the drug [10]. Direct methods, including directly observed therapy (DOT) and measurement of drug concentrations or a biological marker in blood, verify actual tablet intake. Limitations of measuring drug concentrations or a biological marker are related primarily to costs and biological variability [10].

Thus far, no consensus has been reached for the definition of low adherence [6]. The most comprehensive data on real-life statin adherence is provided by fill-rates obtained from pharmacy registry data [11,12]. Such registries are useful for research and community purposes, but they are not available for documentation of statin intake in the individual patient. Low density lipoprotein-cholesterol (LDL-C) has been proposed for measuring adherence to statins [9]. LDL-C response during statin therapy, however, varies considerably between individuals [13], also in populations with high self-reported adherence [14]. This makes it challenging to define reduced adherence across a wide spectrum of individuals. Furthermore, a baseline statin-naïve LDL-C concentration required for a correct assessment of change in LDL-C, is often not available for CVD patients. Self-report questionnaires, such as the Morisky Medication Adherence Scale (MMAS) [15] and the Gehi et al. adherence question [16], are validated tools shown to predict LDL-C levels [17,18] and cardiovascular outcomes in patients being prescribed statins [18,19]. These questionnaires have a potential for clinical use, as they are easy to administer and have established cut-off values. Nevertheless, when compared to robust measures of adherence, such as electronic medication event monitoring systems, self-report questionnaires have been shown to overestimate actual intake [20]. Thus, better tools are needed to accurately and routinely detect low adherence to statin therapy in clinical practice.

We recently developed a fast and reliable assay for direct quantification of atorvastatin and its five major metabolites in blood with liquid chromatography and tandem mass spectrometry (LC-MS/MS) methodology [21]. Based on data from a clinical study of 25 patients with coronary heart disease (CHD) from routine clinical practice, we also established cut-off values that allow discrimination among adherence, partial adherence and non-adherence based on spot measurements of atorvastatin and its metabolites in blood [22]. Importantly, the pharmacokinetic variability (i.e. genetics and drug interactions influencing statin metabolism) is accounted for as the cut-off values are based on the sum of the active drug and its metabolites [22]. The direct method has not previously been compared to blood lipid levels. Furthermore, to what extent self-report adherence measures correspond to directly measured statin adherence is unknown.

The present study therefore aimed to evaluate the relationship between directly measured adherence to atorvastatin, self-reported adherence measures, and the cholesterol levels of outpatients with CHD.

2. Materials and methods

2.1. Design and study population

The present study applies a subpopulation of the cross-sectional NORwegian CORonary (NOR-COR) prevention study which is described in detail elsewhere [23]. In brief, the NOR-COR study comprised 1127 consecutive patients aged 18–80 years undergoing a first or recurrent coronary event from 2011 to 2014 at two Norwegian hospitals (Drammen and Vestfold). All patients underwent a clinical examination and answered a comprehensive self-report questionnaire [23] at a median 16 (range 2–36) months after the event. Blood samples from all patients (N = 542) included at Vestfold hospital were stored for future analyses. Importantly, participants were unaware that their blood samples would later be analyzed with the specific intention of measuring adherence to atorvastatin. In all, 373 patients were included in the present sub-study. Study inclusion criteria were: i) participated in the NOR-COR study in 2014–2015 and having blood samples stored; ii) prescribed atorvastatin at the index event and no information about changes in statin treatment by a physician between the index event and study participation. Participant flow, losses and exclusions are described in Fig. 1.

2.2. Ethics, consent and permission

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in consistence with ICH/Good Clinical Practice. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (2013/1885). All participants gave a written informed consent prior to study start.

2.3. Study assessments

2.3.1. Demographic, clinical and psychosocial factors

We obtained data on demographic, clinical and psychosocial factors retrospectively from hospital medical records at the time of the index coronary event and from the self-report questionnaire, a clinical examination and blood samples at study inclusion [23].

2.3.2. Self-reported measures of adherence

Adherence was assessed using three self-report measures. MMAS-8 [15] and Gehi et al. adherence question [16], as well as a single

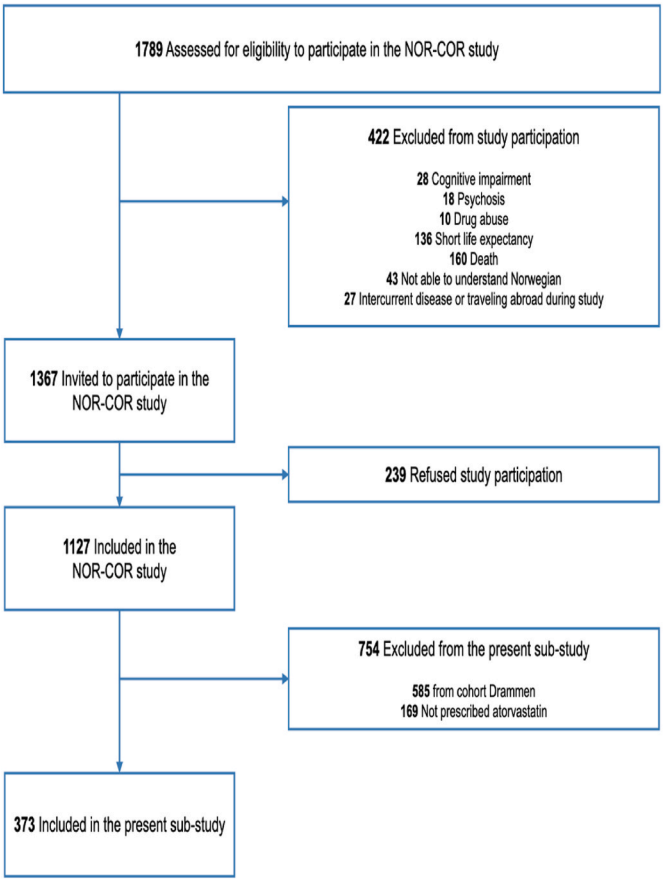

Fig. 1. Study flow chart.

question about statin adherence the past seven days prior to study participation [23]. MMAS-8 is commonly used for measuring medication taking behavior and barriers, and contains 8-items. A score of <6 is considered to be consistent with reduced adherence [15]. The Gehi et al. adherence question is a single question about overall medication adherence [16]: "In the past month, how often did you take your medications as the doctor prescribed?" Possible responses were: "All of the time" (100%), "Nearly all of the time" (90%), "Most of the time" (75%), "About half the time" (50%), or "Less than half of the time" (<50%). Participants replying <90% to the Gehi et al. adherence question were classified with reduced adherence [16]. The statin adherence question asked: "In the past seven days, how often did you take your statin as prescribed?". Possible responses were "Every day", "6/7 days", "5/7 days", "4/7 days" or "<4/7 days". MMAS-8 and the Gehi et al. adherence question have both previously been validated against clinical cardiovascular outcomes using the cut-offs described [18,19]. For the specific statin adherence question, we defined reduced adherence as statin intake <6/7 days.

2.3.3. Direct measurement of adherence to atorvastatin

Venous blood was sampled in EDTA vacutainers. Following centrifugation, the plasma was stored at −70 °C until analysis. Atorvastatin and its major metabolites were quantified using a 2-channel multiplex LC-MS/MS instrument (Transcend II LX-2 TSQ Quantiva, Thermo Fisher Scientific, Waltham, MA, USA) at Oslo University hospital according to the ISO 15189 standard. The assay was specifically designed for measuring adherence as previously described [21]. All analyses of atorvastatin and metabolites fulfilled the acceptance criteria of analytical runs according to the EMA Guideline on bioanalytical method validation [24]. Participants were classified as adherent, partially adherent or non-adherent according to their blood plasma concentrations of atorvastatin and metabolites by our recently developed algorithm [22]. The cut-off for partial adherence (dose-normalized sum of atorvastatin plus metabolites below 0.10 nM/mg) reflects ≥2 consecutively omitted doses with 100% sensitivity and 92% specificity, whereas the cut-off for non-adherence (2-OH atorvastatin acid below 0.014 nmol/L) reflects >3 consecutively omitted doses [22]. There is no established definition for partial adherence when using the self-reported adherence measures. Therefore, participants classified with partial or non-adherence by the direct method, for the purpose of comparing with the self-report measures, were merged and classified as reduced adherence, defined by ≥ 2 doses omitted.

2.4. Statistical analyses

Descriptive data are presented as means (SD) for approximately normally distributed variables and median (IQR) for skewed variables. We assessed differences in blood cholesterol levels between patients classified by the direct method as adherent, partially adherent and non-adherent using one-way ANOVA for continuous variables. Differences in baseline characteristics (adherent *vs.* non-adherent) were assessed using the two-sample T test with adjustment for unequal variances and continuous variables with a marked non-normal distribution not amendable through transformations were tested using the Wilcoxon-Mann-Whitney test. Differences in categorical variables were assessed using Chi-Square tests. Agreement between adherence methods was estimated using unweighted Cohen's kappa (≤0 - no agreement to 1 - perfect agreement) [25] with confidence intervals based on the Fleiss-Cohen-Everitt standard error estimate. Statistical analyses were performed using SPSS version 26.

3. Results

In all, 373 participants prescribed atorvastatin were included in this study (Fig. 1). Baseline characteristics of the participants and the non-participants are presented in Supplementary Table 1. Participants included in this sub-study had higher education, a more favorable cardiovascular risk profile, and less somatic comorbidity, including coronary events prior to the index event, than non-participants.

3.1. Baseline characteristics

Baseline characteristics of participants according to adherence determined by the direct method are shown in Table 1. By the direct method, 344 (92.2%) had high adherence and 29 (7.8%) had reduced adherence. Plasma atorvastatin concentrations according to adherence classification are shown in Supplementary Fig. 1. Patients classified with reduced adherence were more likely to report side effects of cardiovascular drugs than those with normal adherence ($p = 0.007$). Otherwise, there were no significant differences in demographic, clinical or psychosocial characteristics between the groups.

3.2. Adherence and blood LDL cholesterol levels

LDL-C levels were significantly higher in participants classified with reduced adherence compared to those with high adherence when adherence was determined by the direct method, by the specific statin question, and by the Gehi et al. adherence question (Table 2). For MMAS-8, this difference was not significant (Table 2).

Participants classified as adherent by the direct method had significantly lower LDL-C levels (N = 344, mean LDL-C 1.9 [SD 0.6] mmol/L) than those with partial adherence (N = 19, mean LDL-C 2.4 [SD 1.0]

Table 1
Sociodemographic, clinical and psychological characteristics of study participants according to atorvastatin adherence determined by the direct method.

Characteristics	Adherent (N = 344, 92.2%)	Reduced adherence (N = 29, 7.8%)
Sociodemographic factors		
Female, *n* (%)	64 (18.6)	6 (20.7)
Age in years, mean (SD)	63.1 (9.1)	61.4 (9.1)
Low education[a], *n* (%)	224 (65.9)	21 (72.4)
Time since the index coronary event in months, median (IQR)	17 (8.3–28.8)	21 (8.0–29.5)
Clinical factors		
Atorvastatin dose in mg, mean (SD)	64 (20.4)	57 (24.8)
Coronary index diagnosis, *n* (%)		
Acute myocardial infarction	297 (86.3)	24 (82.8)
Stable or unstable angina	47 (13.3)	5 (17.2)
>1 coronary event prior to the index event, *n* (%)	78 (22.7)	7 (24.1)
Charlston comorbidity index, mean (SD)	3.9 (1.7)	3.8 (1.5)
C-reactive protein, median (IQR)	1.3 (0.7–2.7)	1.8 (1.0–2.8)
Systolic blood pressure, mmHg (SD)	137 (17.8)	139 (21.9)
Diabetes mellitus, *n* (%)	41 (11.9)	2 (6.9)
Current smoking, *n* (%)	66 (20.1)	9 (33.3)
Body mass index in kg/m^2, mean (SD)	28.1 (4.4)	28.4 (4.7)
Physical activity < 1 time per week, *n* (%)	47 (14.2)	5 (17.9)
Any self-reported side-effects attributed to their cardiovascular drugs, *n* (%)	73 (21.2)	12 (41.4)
Psychological factors		
Hospital Anxiety and Depression Scale - anxiety sub-scale, mean (SD)	4.7 (3.7)	5.2 (3.5)
Hospital Anxiety and Depression Scale - depression sub-scale, mean (SD)	3.6 (3.2)	3.5 (2.3)
Bergen Insomnia Scale sum[b], median (IQR)	11 (5–20)	8 (6–20)
Type D social inhibition score, mean (SD)	7.7 (5.7)	7.6 (5.7)
Type D negative affectivity score, mean (SD)	6.8 (5.7)	7.5 (7.2)

SD, standard deviation IQR, interquartile range.
[a] Low education was defined as completion of primary and secondary school only.
[b] Bergen insomnia scale: a seven-item self-report inventory designed to assess primary insomnia.

Table 2
Directly measured atorvastatin adherence, self-reported measures of adherence, and blood cholesterol levels.

	Directly measured atorvastatin adherence	Self-reported statin adherence past 7 days	Self-reported medication adherence past month (Gehi)	Morisky Medication Adherence Scale
Reduced adherence[a], *n* (%)	29 (7.8)	19 (5.5)	11 (3.0)	29 (8.4)
LDL-C[b], adherent, mean (SD[c])	1.9 (0.6)	1.9 (0.6)	1.9 (0.6)	1.9 (0.6)
LDL-C, reduced adherence, mean (SD)	2.8 (1.0)	2.8 (1.0)	3.2 (1.1)	2.1 (0.8)
LDL-C adherent *vs.* reduced adherence	$p < 0.001$	$p = 0.001$	$p = 0.004$	$p = 0.07$

[a] Reduced adherence is defined by ≥ 2 doses omitted (merged partial and non-adherence) by the direct method, <6/7 days for self-reported statin adherence, <90% for the Gehi question and a score of <6 on the MMAS-8.
[b] LDL-C, low density lipoprotein-cholesterol.
[c] SD, standard deviation.

mmol/L) and non-adherence (N = 10, mean LDL-C 3.6 [SD 0.6] mmol/L) (adherence *vs.* partial adherence; $p = 0.008$, partial adherence *vs.* non-adherence; $p < 0.001$).

3.3. Relationship between the direct method and self-report adherence measures

The relationships between adherence to atorvastatin determined by the direct method and the self-reported adherence measures are presented in Table 3A-C and illustrated in Fig. 2. In patients classified with reduced adherence by the direct method, 40% reported reduced statin adherence the past week, 32% reported reduced adherence with MMAS-8 and 22% with the Gehi et al. adherence question. In those adherent by the direct method, 96% reported high statin adherence the past week, 95% reported high adherence on MMAS-8 whereas 94% reported high adherence on the Gehi question.

Table 3
Agreement between the statin adherence question and the direct method (A), the Gehi et al. adherence question and the direct method (B) and the 8-item Morisky adherence scale (MMAS-8) and the direct method (C).

(A)		**Direct method** Adherence (*n* = 337)	Reduced adherence (*n* = 25)
Statin adherence past seven days	Adherence (*n* = 343)	328	15
	Reduced adherence (*n* = 19)	9	10
(B)		**Direct method** Adherence (*n* = 340)	Reduced adherence (n = 27)
Gehi et al. adherence question	Adherence (*n* = 356)	335	21
	Reduced adherence (*n* = 11)	5	6
(C)		**Direct method** Adherence (*n* = 323)	Reduced adherence (*n* = 22)
MMAS-8	Adherence (*n* = 316)	301	15
	Reduced adherence (*n* = 29)	22	7

Overall agreement between the direct method and the statin adherence question (Table 3A) was moderate (Cohen's kappa 0.42 (95% CI 0.23 to 0.61)). Among 11 participants with missing data on the self-report measure, four were classified with reduced adherence and seven as adherent by the direct method. Overall agreement between the direct method and the Gehi et al. adherence question was fair (Cohen's kappa 0.29 (0.09–0.48)) (Table 3B). Among six participants with missing data on the self-report measure, two were classified with reduced adherence and four as adherent by the direct method. Overall agreement between the direct method and MMAS (Table 3C) was fair (Cohen's kappa 0.22 (95% CI 0.05 to 0.46)). Among 28 participants with missing data on the self-report measure, seven were classified with reduced adherence, and 21 as adherent by the direct method.

4. Discussion

In this cross-sectional study, the direct method based on atorvastatin plasma concentrations confirmed high self-reported statin adherence in more than 9 out of 10 coronary outpatients. To the contrary, only 20–40% of patients with reduced adherence determined by the direct method were classified accordingly by self-report measures. Even though specific questions about statin adherence better reflected directly measured atorvastatin adherence than more general adherence questions, overall agreement between the direct method and the self-report measures was only fair to moderate. We found a graded, inverse relationship between the direct method of adherence to atorvastatin and blood cholesterol levels.

Statins exert their beneficial effects on CVD primarily by reducing harmful circulating lipoproteins, in particular LDL-C [26]. It was therefore expected that patients with normal adherence based on atorvastatin and metabolites concentration in blood had lower LDL-C levels than those with partial and non-adherence. After all, "*drugs don't work in patients who don't take them*" (C Everett Koop, MD, US Surgeon General, 1985). To the best of our knowledge, this is the first study applying a direct method [21] and algorithm [22] that allows discrimination among adherence, partial adherence and non-adherence to the most commonly used statin [27]. The patients classified as partially adherent by the direct test, with 2 or 3 subsequently missed doses, had significantly higher blood levels of LDL-C than those classified as adherent, and significantly lower LDL-C than those classified as non-adherent (>3 missed doses). This provides a biological rationale for the present direct classification including an intermediate group with partial adherence. This may indicate that the direct test also identifies patients with borderline adherence, omitting doses habitually.

The direct method to determine adherence to atorvastatin is based on a clinical pharmacokinetic study [22]. Importantly, the derived cut-off values are dose-normalized and thus account for individual differences in the atorvastatin metabolism by summarizing parent drug and metabolites [22]. Two previous studies have applied an LC–MS/MS assay to determine adherence to atorvastatin [28,29]. The classification of adherence in these studies, based solely on the presence or absence of atorvastatin in blood [28,29] has several limitations as described elsewhere [21,22]. The current LC-MS/MS assay detects blood levels of atorvastatin weeks after intake due to the drug half-life and instrument sensitivity [21,22]. One balanced cut off value, only, for atorvastatin will thus misclassify a significant number of adherent and non-adherent patients, and render the test less useful, also for the communication between physician and patient. Of major importance, this dichotomous categorization does not account for the inverse, and graded, relationship between adherence and mortality [5]. Thus, patients who are partially adherent may be at higher risk of discontinuing their treatment in the future and subsequently at risk of poorer outcomes.

In line with our results, previous studies on other cardiovascular drugs have shown low agreement between self-reported adherence measures and more robust measures of adherence such as electronic

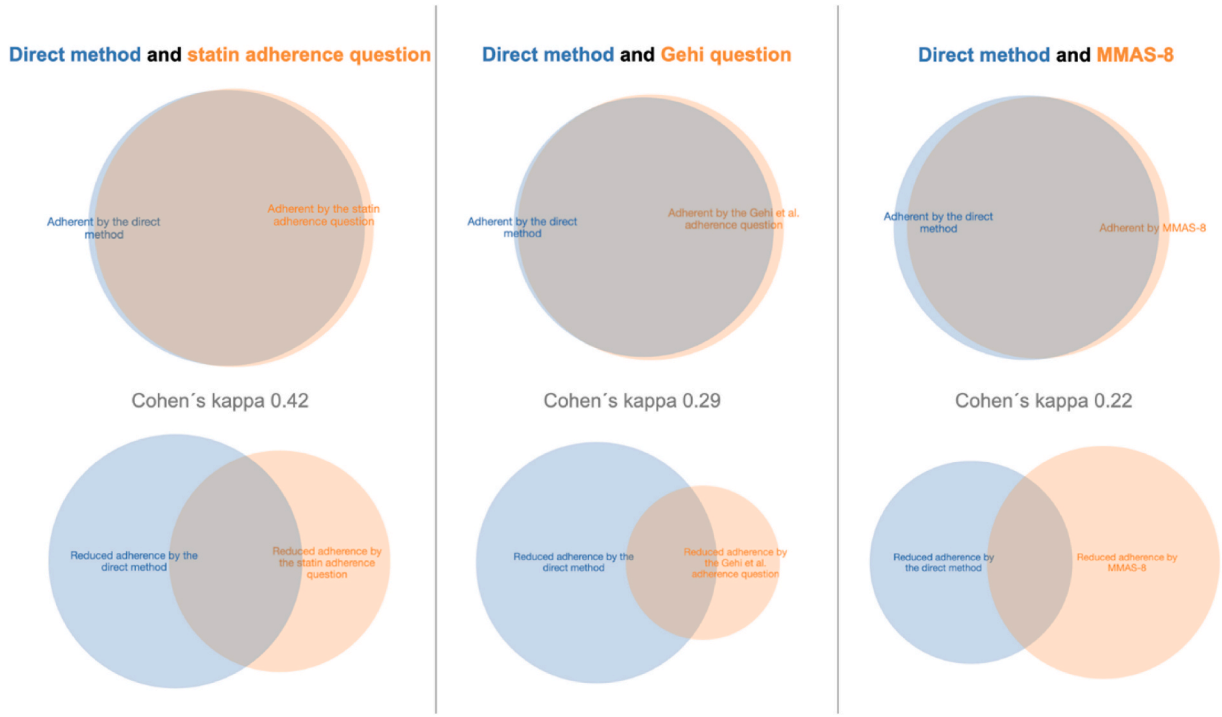

Fig. 2. Venn diagrams illustrating agreement between the direct method and self-report measures of adherence.

medication event monitoring systems and measurement of drug concentrations [20,30]. Self-report questionnaires rely heavily on patients' recall, and for statins they have been shown to significantly overestimate actual intake [31]. We found an increasing agreement between the direct method with increasing specificity of the questions in the self-report measure. The poorest agreement was observed with for MMAS-8 which is expected as this is a general measure of adherence behavior. A too low cut-off value for reduced adherence for the direct method may also influence the agreement score, confer the intention of the algorithm to avoid that adherent patients are misclassified as partially adherent [22]. Nevertheless, the vast majority of patients classified as adherent by the direct method were also classified as adherent by the self-report measures suggesting that most of these patients provide an accurate record upon questioning. In contrast, only 40% classified with reduced adherence by the direct method reported reduced statin adherence the past 7 days prior to study participation. An even lower proportion of these patients reported reduced adherence with the general adherence measures. This finding may be related to differences in length of the periods to which the self-report questionnaires refer to. Furthermore, the MMAS-8 only contains 2 times assessing adherence within a concrete time interval which likely contributes to a lower agreement. Nonetheless, the poor performance of self-report measures to identify the high-risk sub-group with reduced adherence accords to previous comparisons with electronic monitoring systems [20]. It is concerning and emphasizes the need for valid methods to measure statin adherence in clinical practice.

Self-perceived side effect of cardiovascular drugs was the only potentially modifiable clinical factor that significantly discriminated patients with reduced adherence from to those with high adherence. Accordingly, a recent randomized trial with long-term follow up, found statin-specific side effects to be the most important predictor of poor adherence [32].

Although most patients with high adherence may be identified by asking direct questions regarding statin adherence most of those with reduced adherence will not. After further validation, the direct method may prove to be a useful tool in clinical practice to identify patients with reduced adherence to statins, at risk of future treatment discontinuation and poor prognosis. Moreover, an objective confirmation of reduced adherence to statin therapy may provide an entry into a careful discussion between the patient and the healthcare provider about adherence, causes of reduced adherence and ways to improve it.

4.1. Strengths and limitations

None of the study participants were aware that their blood samples would be analyzed for statin adherence at the time of sampling and completion of the questionnaires. Thus, the risk of white coat adherence and biased reporting of adherence is low. It is a strength that the algorithm of the present direct method translates the pharmacokinetics of atorvastatin into terms and levels of clinical adherence that correspond to the biological statin effects (i.e. blood cholesterol levels). However, before the method may be used in clinical practice eventually, further large-scale validation of the algorithm is needed as this may reveal rare pharmacokinetic and pharmacodynamic factors that should be accounted for [22]. The comprehensive dataset with several self-report measures of adherence and potential determinants of reduced adherence are other important strengths of the study. Adherence to atorvastatin determined by the direct method was high (92%) in the present cohort and limited to patients <80 years being prescribed atorvastatin and in whom the treating physician had not discontinued or changed statin treatment during the median 16 months period prior to study participation. Furthermore, the study participants had significantly lower LDL-C and fewer previous CHD-events than the non-participants (Supplementary Table 1), indicating lower adherence among the non-participants. The low prevalence of reduced adherence in this selected population may potentially affect the agreement scores between the self-report measures and the direct method. Even though there were few missing data on the self-reported measures, this may also bias the kappa agreement scores. There was a graded relationship between the direct method and blood LDL-C levels. However, it is uncertain to what extent a spot measurement of atorvastatin in blood reflects long-term persistence to statin treatment. Ideally, we would assess directly measured adherence in relation to change in LDL-C from baseline to follow up. However, this was not possible due to lack of baseline

blood lipid levels in most participants. The direct method measures adherence only in the days prior to blood sampling. It is therefore possible that some poorly adherent patients not detected by the direct method may be detected by self-report measures. This may be investigated in future studies combining self-report measures, pharmacy registry data with the direct method. Finally, although LDL-C is causal for the development of atherosclerotic CVD, the association between level of adherence measured directly and hard clinical outcomes remain to be studied.

4.2. Conclusions

There was a graded, inverse relationship between directly determined adherence to atorvastatin and cholesterol levels in blood. Most patients classified with reduced adherence to atorvastatin by plasma drug concentrations do not report reduced adherence on self-report questionnaires. Specific questioning about statin adherence was more likely to agree with the direct method than general adherence measures, but the agreement score was still only fair to moderate. The direct method may be a useful tool to further identify reduced statin adherence and provide an entry point into a dialogue with patients about clinical decisions of treatment.

Declaration of competing interest

L.G. reports having received modest lecture fees from Astra Zeneca, Amgen, and Sanofi, outside the submitted work. E.G. reports having received modest lecture fees from BMS and Boehringer Ingelheim, outside the submitted word. J.M. reports having received modest lecture fees from Astra Zeneca, Amgen and Bayer, outside the submitted work. Otherwise, the authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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CRediT authorship contribution statement

Oscar Kristiansen: Writing – original draft, Investigation, Formal analysis, Visualization. **Elise Sverre:** Project administration, Writing – review & editing, Investigation, Data curation. **Kari Peersen:** Investigation, Writing – review & editing. **Morten Wang Fagerland:** Formal analysis, Visualization, Writing – review & editing. **Erik Gjertsen:** Conceptualization, Supervision. **Lars Gullestad:** Conceptualization, Methodology, Writing – review & editing. **Joep Perk:** Conceptualization, Methodology, Writing – review & editing. **Toril Dammen:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Einar Husebye:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Nils Tore Vethe:** Methodology, Writing – review & editing, Supervision. **John Munkhaugen:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.09.020.

References

[1] R. Collins, C. Reith, J. Emberson, et al., Interpretation of the evidence for the efficacy and safety of statin therapy, Lancet 388 (2016) 2532–2561, https://doi.org/10.1016/S0140-6736(16)31357-5.
[2] C. Cholesterol Treatment Trialists, C. Baigent, L. Blackwell, et al., Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials, Lancet 376 (2010) 1670–1681, https://doi.org/10.1016/S0140-6736(10)61350-5.
[3] L.D. Colantonio, R.S. Rosenson, L. Deng, et al., Adherence to statin therapy among US adults between 2007 and 2014, J Am Heart Assoc 8 (2019), e010376, https://doi.org/10.1161/JAHA.118.010376.
[4] M.A. De Vera, V. Bhole, L.C. Burns, et al., Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review, Br. J. Clin. Pharmacol. 78 (2014) 684–698, https://doi.org/10.1111/bcp.12339.
[5] F. Rodriguez, D.J. Maron, J.W. Knowles, et al., Association of statin adherence with mortality in patients with atherosclerotic cardiovascular disease, JAMA Cardiol 4 (2019) 206–213, https://doi.org/10.1001/jamacardio.2018.4936.
[6] H. Drexel, A.J.S. Coats, I. Spoletini, et al., An expert opinion paper on statin adherence and implementation of new lipid-lowering medications by the ESC Working Group on Cardiovascular Pharmacotherapy: barriers to be overcome, Eur Heart J Cardiovasc Pharmacother 6 (2020) 115–121, https://doi.org/10.1093/ehjcvp/pvz079.
[7] M.L. van Driel, M.D. Morledge, R. Ulep, et al., Interventions to improve adherence to lipid-lowering medication, Cochrane Database Syst. Rev. 12 (2016) Cd004371, https://doi.org/10.1002/14651858.CD004371.pub4.
[8] Authors/Task Force M, Guidelines ESCCfP and Societies ESCNC, 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Atherosclerosis 290 (2019) 140–205, https://doi.org/10.1016/j.atherosclerosis.2019.08.014.
[9] S.M. Grundy, N.J. Stone, A.L. Bailey, et al., AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American college of cardiology/American heart association task force on clinical practice guidelines, J. Am. Coll. Cardiol. (2018), https://doi.org/10.1016/j.jacc.2018.11.003, 2018.
[10] L. Osterberg, T. Blaschke, Adherence to medication, N. Engl. J. Med. 353 (2005) 487–497, https://doi.org/10.1056/NEJMra050100.
[11] P.M. Ho, C.L. Bryson, J.S. Rumsfeld, Medication adherence: its importance in cardiovascular outcomes, Circulation 119 (2009) 3028–3035, https://doi.org/10.1161/CIRCULATIONAHA.108.768986.
[12] V. Kini, P.M. Ho, Interventions to improve medication adherence: a review, Jama 320 (2018) 2461–2473, https://doi.org/10.1001/jama.2018.19271.
[13] B.W. Karlson, O. Wiklund, M.K. Palmer, et al., Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: results from VOYAGER, Eur Heart J Cardiovasc Pharmacother 2 (2016) 212–217, https://doi.org/10.1093/ehjcvp/pvw006.
[14] D. Bacquer, D. Smedt, Ž. Reiner, et al., Percentage low-density lipoprotein-cholesterol response to a given statin dose is not fixed across the pre-treatment range: real world evidence from clinical practice: data from the ESC-EORP EUROASPIRE V Study, Eur J Prev Cardiol 27 (2020) 1630–1636, https://doi.org/10.1177/2047487319874898.
[15] D.E. Morisky, A. Ang, M. Krousel-Wood, et al., Predictive validity of a medication adherence measure in an outpatient setting, J. Clin. Hypertens. 10 (2008) 348–354, https://doi.org/10.1111/j.1751-7176.2008.07572.x.
[16] A. Gehi, D. Haas, S. Pipkin, et al., Depression and medication adherence in outpatients with coronary heart disease: findings from the Heart and Soul Study, Arch. Intern. Med. 165 (2005) 2508–2513, https://doi.org/10.1001/archinte.165.21.2508.
[17] M. Bermingham, J. Hayden, I. Dawkins, et al., Prospective analysis of LDL-C goal achievement and self-reported medication adherence among statin users in primary care, Clin. Therapeut. 33 (2011) 1180–1189, https://doi.org/10.1016/j.clinthera.2011.07.007.
[18] A.K. Gehi, S. Ali, B. Na, et al., Self-reported medication adherence and cardiovascular events in patients with stable coronary heart disease: the heart and soul study, Arch. Intern. Med. 167 (2007) 1798–1803, https://doi.org/10.1001/archinte.167.16.1798.
[19] M.A. Krousel-Wood, P. Muntner, T. Islam, et al., Barriers to and determinants of medication adherence in hypertension management: perspective of the cohort study of medication adherence among older adults, Med. Clin. 93 (2009) 753–769, https://doi.org/10.1016/j.mcna.2009.02.007.
[20] M. El Alili, B. Vrijens, J. Demonceau, et al., A scoping review of studies comparing the medication event monitoring system (MEMS) with alternative methods for measuring medication adherence, Br. J. Clin. Pharmacol. 82 (2016) 268–279, https://doi.org/10.1111/bcp.12942.
[21] N.T. Vethe, J. Munkhaugen, A.M. Andersen, et al., A method for direct monitoring of atorvastatin adherence in cardiovascular disease prevention: quantification of

the total exposure to parent drug and major metabolites using 2-channel chromatography and tandem mass spectrometry, Ther. Drug Monit. 41 (2019) 19–28, https://doi.org/10.1097/FTD.0000000000000578.
[22] O. Kristiansen, N.T. Vethe, M.W. Fagerland, et al., A novel direct method to determine adherence to atorvastatin therapy in patients with coronary heart disease, Br. J. Clin. Pharmacol. (2019), https://doi.org/10.1111/bcp.14122.
[23] J. Munkhaugen, E. Sverre, K. Peersen, et al., The role of medical and psychosocial factors for unfavourable coronary risk factor control, Scand. Cardiovasc. J. 50 (2016) 1–8, https://doi.org/10.3109/14017431.2015.1111408.
[24] Bioanalytical Method Validation, 2020. https://www.ema.europa.eu/en/bioanalytical-method-validation. (Accessed 22 November 2020).
[25] M.L. McHugh, Interrater reliability: the kappa statistic, Biochem. Med. 22 (2012) 276–282.
[26] B.A. Ference, H.N. Ginsberg, I. Graham, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (2017) 2459–2472, https://doi.org/10.1093/eurheartj/ehx144.
[27] The Norwegian Prescription Database, 2020. http://norpd.no/Prevalens.aspx. (Accessed 22 November 2020).
[28] T.P. Ryan, R.D. Morrison, J.J. Sutherland, et al., Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring, PloS One 12 (2017), e0185471, https://doi.org/10.1371/journal.pone.0185471.
[29] J.J. Sutherland, R.D. Morrison, C.D. McNaughton, et al., Assessment of patient medication adherence, medical record accuracy, and medication blood concentrations for prescription and over-the-counter medications, JAMA network open 1 (2018), e184196, https://doi.org/10.1001/jamanetworkopen.2018.4196.
[30] A. Pandey, F. Raza, A. Velasco, et al., Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension, J Am Soc Hypertens 9 (2015) 420–426, https://doi.org/10.1016/j.jash.2015.04.004, e422.
[31] C.W. Cheng, K.S. Woo, J.C. Chan, et al., Assessing adherence to statin therapy using patient report, pill count, and an electronic monitoring device, Am. J. Health Syst. Pharm. 62 (2005) 411–415, https://doi.org/10.1093/ajhp/62.4.0411.
[32] D. Huber, C. Wiken, R. Henriksson, et al., Statin treatment after acute coronary syndrome: adherence and reasons for non-adherence in a randomized controlled intervention trial, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-48540-3.

SUPPLEMENTARY MATERIAL PAPER 2

Paper 2 – Supplementary table 1 – Baseline characteristics of non-participants and participants in the present sub-study.

Characteristic	**Non-participants (N=754, 66.9%)**	**Present sub-study (N=373, 33.1%)**	***p*-Value**
Sociodemographic factors			
Female, *n* (%)	169 (22.4)	70 (18.8)	0.105
Age in years, mean (SD)	62.0 (9.8)	60.9 (9.2)	0.054
Low education, *n* (%)	549 (72.8)	245 (65.7)	0.030
Time since index event, median (IQR)	15.0 (7-25)	17.0 (9-28.5)	0.012
Clinical factors			
Coronary index diagnosis, n (%)			
Acute myocardial infarction	573 (76.0)	321 (86.1)	<0.001
Stable or unstable angina	181 (24.0)	52 (13.9)	
>1 coronary event prior to the index event, % (*n*)	254 (33.7)	85 (22.8)	0.001
Low density lipoprotein cholesterol in mmol/L, mean (SD)	2.2 (0.8)	1.9 (0.7)	0.001
Charlston comorbidity score, mean (SD)	4.2 (1.5)	3.9 (1.3)	0.006
C-reactive protein, median (IQR)	1.7 (0.9-3.0)	1.4 (0.6-2.7)	0.010
Systolic blood pressure, mmHg (SD)	139 (19.6)	137 (18.1)	0.118
Diabetes, *n* (%)	149 (19.8)	43 (11.5)	<0.001
Current smoking, *n* (%)	156 (21.5)	75 (20.1)	0.800
Body mass index in kg/m^2, mean (SD)	28.9 (4.6)	28.1 (4.4)	0.003
Physical activity <1 time per week, *n* (%)	146 (19.8)	52 (13.9)	0.023
Any self-reported side-effects attributed to their cardiovascular drugs, *n* (%)	207 (27.5)	85 (22.8)	0.113
Psychological factors			
Hospital Anxiety and Depression Scale - anxiety sub-scale score, mean (SD)	4.8 (3.8)	4.7 (3.7)	0.833

Hospital Anxiety and Depression Scale - depression sub-scale score, mean (SD)	4.0 (3.3)	3.6 (3.1)	0.052
Bergen Insomnia Scale[b] sum, mean (SD)	14.2 (11.0)	13.4 (10.4)	0.281
Type D social inhibition score, mean (SD)	7.5 (5.6)	7.7 (5.7)	0.432
Type D negative affectivity score, mean (SD)	7.1 (5.9)	6.8 (5.9)	0.459

SD: standard deviation, IQR: interquartile range

[a]Low education was defined as completion of primary and secondary school only.

[b]Bergen insomnia scale: a seven-item self-report inventory designed to assess primary insomnia.

PAPER 3:

Oscar Kristiansen, Nils Tore Vethe, Kari Peersen,
Morten Wang Fagerland, Elise Sverre, Elena Prunés Jensen,
Morten Lindberg, Erik Gjertsen, Lars Gullestad,
Joep Perk, Toril Dammen, Stein Bergan, Einar Husebye,
Jan Erik Otterstad, John Munkhaugen

Effect of atorvastatin on muscle symptoms in coronary heart disease patients with self-perceived statin muscle side effects: a randomized, double-blinded crossover trial

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ORIGINAL ARTICLE
Prevention and epidemiology

Effect of atorvastatin on muscle symptoms in coronary heart disease patients with self-perceived statin muscle side effects: a randomized, double-blinded crossover trial

Oscar Kristiansen [1,2]*, Nils Tore Vethe [3], Kari Peersen[4], Morten Wang Fagerland [5], Elise Sverre [1,2], Elena Prunés Jensen[6], Morten Lindberg [7], Erik Gjertsen [1], Lars Gullestad[8,9,10], Joep Perk[11], Toril Dammen[2], Stein Bergan [3], Einar Husebye[1], Jan Erik Otterstad[4], and John Munkhaugen[1,2]

[1]Department of Medicine, Drammen Hospital, Vestre Viken Hospital Trust, Dronninggata 28, Drammen 3004, Norway; [2]Department of Behavioural Sciences in Medicine, Faculty of Medicine, University of Oslo, Domus Medica, Sognsvannsveien 9, Oslo 0372, Norway; [3]Department of Pharmacology, Oslo University Hospital, Sognsvannsveien 20, Oslo 0372, Norway; [4]Department of Cardiology, Vestfold Hospital Trust, Halfdan Wilhelmsens alle 17, Tønsberg 3103, Norway; [5]Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Domus Medica, Gaustad, Sognsvannsveien 9, Oslo 0372, Norway; [6]Department of Laboratory Medicine, Vestre Viken Hospital Trust, Dronninggata 28, Drammen 3004, Norway; [7]Central Laboratory, Vestfold Hospital Trust, Halfdan Wilhelmsens alle 17, Tønsberg 3103, Norway; [8]Department of Cardiology, Oslo University Hospital Rikshospitalet, Sognsvannsveien 20, 0372, Oslo, Norway; [9]Institute of Clinical Medicine, University of Oslo, Sognsvannsveien 20, Oslo 0372, Norway; [10]KG Jebsen Cardiac Research Centre, Oslo University Hospital, Postbox 4956 Nydalen, Oslo 0424, Norway; and [11]Department of Cardiology, Public Health Department, Linnaeus University, Kalmar 391 82, Sweden

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Aims To estimate the effect of atorvastatin on muscle symptom intensity in coronary heart disease (CHD) patients with self-perceived statin-associated muscle symptoms (SAMS) and to determine the relationship to blood levels of atorvastatin and/or metabolites.

Methods and results A randomized multi-centre trial consecutively identified 982 patients with previous or ongoing atorvastatin treatment after a CHD event. Of these, 97 (9.9%) reported SAMS and 77 were randomized to 7-week double-blinded treatment with atorvastatin 40 mg/day and placebo in a crossover design. The primary outcome was the individual mean difference in muscle symptom intensity between the treatment periods, measured by visual-analogue scale (VAS) scores. Atorvastatin did not affect the intensity of muscle symptoms among 71 patients who completed the trial. Mean VAS difference (statin-placebo) was 0.31 (95% CI: -0.24 to 0.86). The proportion with more muscle symptoms during placebo than atorvastatin was 17% ($n = 12$), 55% ($n = 39$) had the same muscle symptom intensity during both treatment periods whereas 28% ($n = 20$) had more symptoms during atorvastatin than placebo (confirmed SAMS). There were no differences in clinical or pharmacogenetic characteristics between these groups. The levels of atorvastatin and/or metabolites did not correlate to muscle symptom intensity among patients with confirmed SAMS (Spearman's rho ≤0.40, for all variables).

Conclusion Re-challenge with high-intensity atorvastatin did not affect the intensity of muscle symptoms in CHD patients with self-perceived SAMS during previous atorvastatin therapy. There was no relationship between muscle symptoms

* Corresponding author. Tel: +47 47682535, Email: osckri@vestreviken.no

and the systemic exposure to atorvastatin and/or its metabolites. The findings encourage an informed discussion to elucidate other causes of muscle complaints and continued statin use.

Keywords Statin-associated muscle symptoms • Coronary heart disease • Atorvastatin • Crossover trial • Placebo-controlled

Introduction

There is firm evidence that statins prevent cardiovascular events, with low rates of serious adverse events.[1–3] Nevertheless, 19% of those using statins for secondary cardiovascular disease prevention in the UK discontinue their treatment within the 1st year, increasing to 26% at 2 years.[4] The principle reason for poor adherence is statin-associated muscle symptoms (SAMS),[5] a heterogeneous group of muscle complaints occurring upon initiation of treatment or an increase in dose.[5] As poor adherence to statin therapy is associated with increased morbidity and mortality,[6] SAMS represent a major challenge in the prevention of cardiovascular disease.

In observational studies, SAMS are frequently reported (10–25%) and statin-treated individuals are more likely to report muscle symptoms than those who are not using a statin.[7–10] In contrast, the randomized trials have not found significant differences in the prevalence of muscle side effects between statin and placebo.[11–13] Strict entry criteria in these trials have been suggested as possible explanations for this discrepancy.[5,14] Patients treated with statins may expect muscle side effects, and therefore report more muscle symptoms than untreated patients, the so-called 'nocebo effect'. Double-blinded crossover trials, exposing participants to both active treatment and placebo in random order, are needed to confirm whether side effects are drug related or not.[2] A small proof-of-concept crossover study[15] and two larger trials designed to test the effect of non-statin therapies[16] and coenzyme Q10[17] in selected patients with SAMS reported conflicting results. Thus, the effect of statin therapy on muscle symptoms remains to be settled.

Although pathophysiological mechanisms[18] and clinical diagnostic algorithms for SAMS have been proposed,[19] it remains unclear how statins produce muscle symptoms, and reliable biomarkers for SAMS are requested.[5] Elevated levels of statin metabolites have been proposed as underlying mechanisms of SAMS.[20] In particular, the lactone metabolites of statins have been associated with muscle toxicity *in vitro* and *in vivo*.[21–23] The relationship between muscle complaints and the exposure to statin metabolites has not previously been studied under randomized, placebo-controlled conditions.

This study aimed to estimate the effect of atorvastatin on muscle symptom intensity in patients with self-perceived SAMS after a coronary heart disease (CHD) event and to determine the relationship between SAMS and the levels of atorvastatin and its metabolites in blood plasma.

Methods

Trial oversight

MUscle Side-Effects of atorvastatin in coronary patients (MUSE) was a multi-centre, randomized, double-blinded, placebo-controlled, two-period crossover trial designed to test the effect of atorvastatin 40 mg/day on muscle symptom intensity.[24] The crossover design allows both within- and between-patient comparisons of muscle symptoms reported on placebo and atorvastatin and requires a lower number of patients than a parallel group design. The protocol is available at ClinicalTrials.gov. There were no significant changes of methods or outcomes after trial commencement. The trial was reported according to the CONSORT guidelines[25] and registered in the European Clinical Trials Database (2018-004261-14) and at ClinicalTrials.gov (NCT03874156), prior to inclusion of the first patient. The trial complies with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics (2018/2302), the Norwegian Medicines Agency (18/17102-16), and the local data protection officers. All patients gave written informed consent. The trial was monitored by research cardiologists.

Participants

All patients discharged with a first or recurrent CHD event between 2016 and 2019 were retrospectively identified through hospital discharge lists from two secondary care hospitals. The catchment area to the hospitals corresponds to 7.4% of the Norwegian population and is representative of Norwegian geography, economy, age distribution, morbidity, and mortality.[26] All patients underwent a standardized telephone interview to reveal whether they had (i) subjective SAMS during ongoing atorvastatin therapy or (ii) previous muscle symptoms that had led to discontinuation of atorvastatin. All patients with self-perceived SAMS were invited to the outpatient clinics for an evaluation of entry criteria and a detailed interview by two study cardiologists before randomization. The interview focused on the temporal association between muscle symptoms and initiation and discontinuation of the statin treatment. Patients who had transitory muscle complaints and who expressed uncertainty as to whether the symptoms were actually caused by the statin, were excluded from the study at baseline (*Figure 1*). An overview of patients excluded prior to the telephone interview is provided in Supplementary material online, *Appendix Figure S1*. The eligibility criteria are described in detail elsewhere.[24] An age and sex-matched control group of CHD patients reporting no history of SAMS despite atorvastatin ≥40 mg, and no other ongoing muscular complaints, was assigned to 7 weeks open-label treatment of atorvastatin 40 mg/day to compare blood plasma concentrations of atorvastatin and metabolites.

Interventions

Participants were randomly assigned by two study cardiologists to atorvastatin in treatment period one, followed by placebo in treatment period two, or vice-versa (AB-BA crossover design). A morning dose of 40 mg/day was chosen, as this is a high-intensity statin treatment frequently used by patients with CHD. Each treatment period was preceded by a 1-week pharmacological washout and lasted for 7 weeks or until intolerable muscle symptoms occurred. The length of the treatment period was chosen on the basis of two observational studies[9,27] indicating that SAMS appear median 2 and 4 weeks after re-challenge and initiation of statin treatment, respectively. The washout periods corresponded to more than 10 half-lives of atorvastatin and its metabolites in the systemic

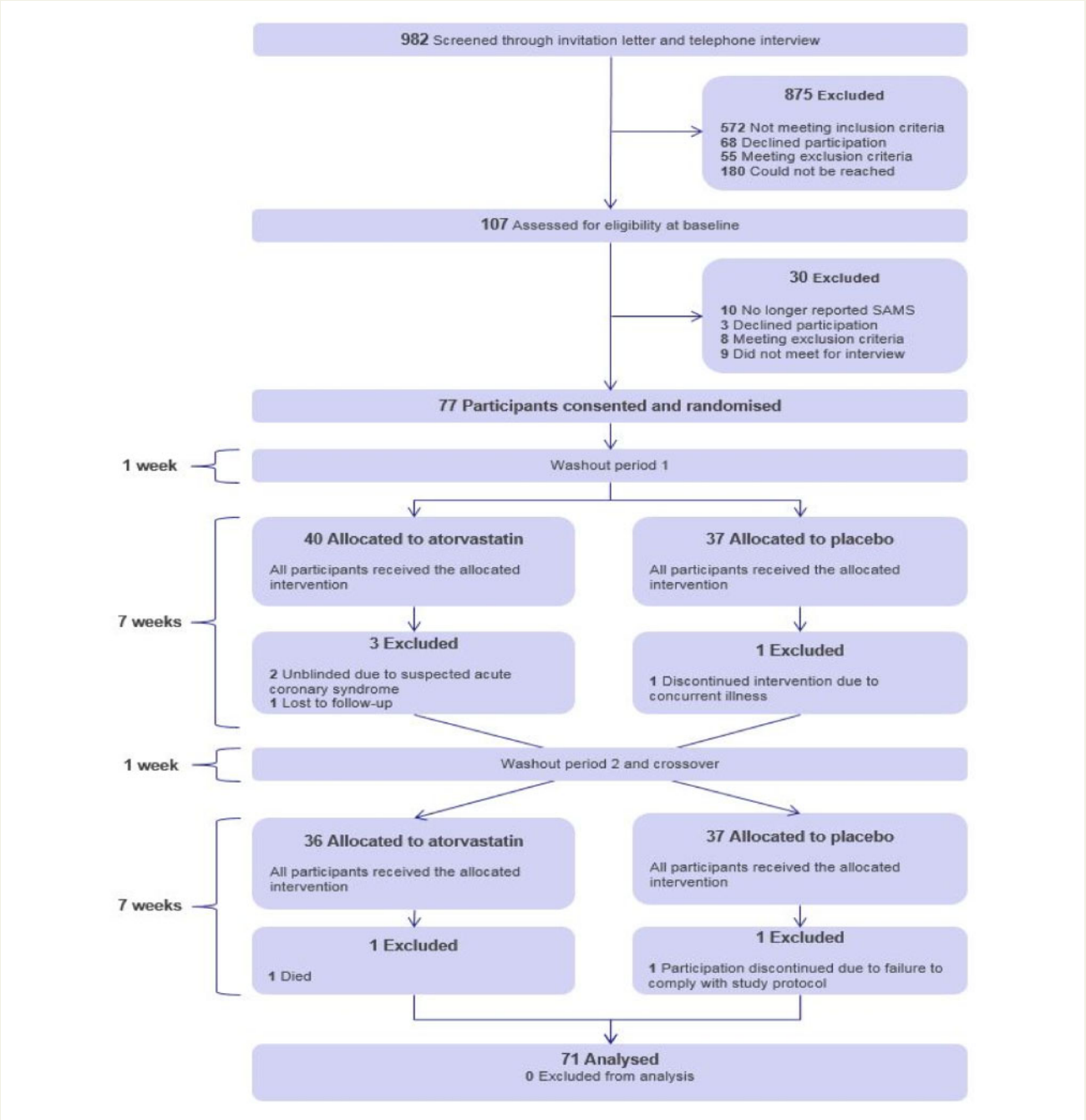

Figure 1 CONSORT flow diagram.

circulation.[28] The primary endpoint was analysed on the basis of symptom intensity in the final 3 weeks of each treatment period. Thus, the risk of carryover effects was minimized as SAMS improve after a median of 2 weeks following treatment discontinuation.[27]

Data collection

Clinical data were collected at baseline from hospital medical records and a self-report questionnaire. Muscle symptom (pain, weakness, tenderness, stiffness, or cramps) intensity was registered weekly in a patient diary using a 0 (no symptoms) to 10 (worst imaginable) visual-analogue scale (VAS). Blood samples for measurement of atorvastatin and metabolites concentration in plasma were obtained immediately prior to the next scheduled dose (C_0, trough concentration) and 2 h after observed tablet intake (C_2, reflecting the peak concentration according to the pharmacokinetic profile of the drug) on the last day of each treatment period. Food intake was allowed prior to collection of C_0 samples but participants fasted until C_2 samples were collected.[28] The blood samples were handled and analysed as previously described.[29] Relevant sequence variants in the *SLCO1B1* (*5, c521T>C, rs4149056), *CYP3A4* (*22,

rs35599367), and *CYP3A5* (*3, rs776746) genes were analysed in baseline blood samples (Light Cycler® 480, Roche Diagnostics). All participants and study personnel were blinded to the results of all laboratory tests including low-density lipoprotein cholesterol and creatine kinase (CK) levels during the study period. Adherence was measured by pill counts in returned containers as well as by drug measurement directly in blood[30] at the end of each treatment period.

Outcomes

The primary outcome was the individual difference in muscle symptom intensity between treatment periods, measured by mean VAS scores during the last 3 weeks of each treatment period. This outcome was chosen to (i) ensure steady-state concentrations of atorvastatin, (ii) maximize the likelihood for the symptoms reported to be truly related to the current (and not previous) treatment period, and (iii) ensure sufficiently long treatment periods for SAMS to appear/disappear. Key secondary outcomes were (i) the proportion with confirmed SAMS, defined as a 25% higher individual mean VAS-score during the treatment period on atorvastatin vs. placebo, and ≥1 cm absolute difference, as this has been regarded as a clinically relevant difference in a validation study of the VAS scale,[31] (ii) the correlation between individual differences in mean muscle symptom intensity and levels of atorvastatin and metabolites among patients with confirmed SAMS, (iii) diagnostic properties of atorvastatin and metabolites for the diagnosis of confirmed SAMS, and (iv) difference between levels of atorvastatin and metabolites in patients with failing placebo test for connecting SAMS to atorvastatin (i.e. non-SAMS) and the control group. Further details on all pre-specified outcomes are available in the statistical analysis plan (SAP) (see Supplementary material online, *Appendix S1*).

Randomization

Participants were randomized by an independent statistician in a 1:1 ratio to a double (i.e. participants, providers, those assessing outcomes) blinded treatment sequence of atorvastatin and matching placebo using an electronic randomization system. Block sizes of four and six in random order, stratified according to centre and previous atorvastatin discontinuation were used. Tablets were encapsulated with identical appearance for atorvastatin and placebo. The containers were collected at the end of each treatment period to avoid participants attempting to compare capsules.

Statistical analyses

Sample size calculations are based on the ability to detect a 1 cm difference in the VAS score between the treatment periods on atorvastatin and placebo.[31] With $n = 68$, we will have 90% power to detect a difference of 1.0 (SD = 2.5) (one-sample *T*-test) and 80% power to detect a difference of 40% SAMS under statins vs. 15% SAMS under placebo (the McNemar test). To account for missing data due to drop-outs or and protocol deviations, we aimed to include 80 patients. All analyses were specified prior to database lock, except where noted, and are described in detail in the SAP. The primary outcome was estimated as the predictive overall margin [95% confidence interval (CI)] 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 primary analysis was performed on the full analysis set. A secondary analysis was performed on the per-protocol set. A 95% CI for the proportion of confirmed SAMS was estimated with the Wilson score confidence interval.[32] The correlations between differences in muscular symptom intensity and levels of atorvastatin and metabolites among patients with confirmed SAMS were estimated with Spearman rank correlation coefficients, with 95% CIs estimated by the Bonett–Wright approximation.[33] 95% CI of per cent estimates are given in succeeding brackets. Receiver operating characteristics curves and measures of diagnostic accuracy were used to identify cut-off values of metabolite concentrations that can discriminate confirmed SAMS from other muscle symptoms. The comparison of levels of atorvastatin and metabolites between non-SAMS participants and the control group were performed with two-sample *T*-tests with adjustment for unequal variances. A senior statistician, blinded to participants' treatment sequence, performed all analyses using Stata/SE 16.0 (StataCorp LLC, College Station, TX, USA) and Matlab R2014a (The MathWorks, Inc.).

Results

Participant flow, losses, and exclusions are shown in *Figure 1*. Among 982 atorvastatin-treated patients telephoned for assessment of eligibility (82% response rate), 875 were ineligible, most commonly due to no history of self-perceived SAMS. Only one patient had self-perceived SAMS among those who declined to participate. Ninety-seven patients (9.9%) reported SAMS at the baseline interview. Of these, 77 (79%) were randomized in March and August 2019 and 71 completed the trial in June and December 2019. These participants constitute the full analysis set. One patient with significantly elevated blood levels of alanine aminotransferase at the end of the atorvastatin period, and one patient with atorvastatin present in blood plasma during the placebo period were excluded from the full analysis set, leaving 69 participants eligible for the per-protocol analysis of SAMS and atorvastatin exposure. Overall, adherence as measured by pill counts was high with a mean proportion of days covered of 99% (range 91–100%). Adherence was also confirmed by the direct method. There were no missing data on muscle symptom intensity, hospital medical records, or blood samples. Less than 5% of the data from patient questionnaires were missing.

Baseline characteristics

Characteristics were well balanced between treatment sequences (*Table 1*). There was no information in hospital records about previous statin discontinuation (i.e. de-challenge) and repetitive re-challenge tests among study participants. Nineteen patients (27%) had tried ≥2 statins prior to study start. Except for ezetimibe, no other lipid-lowering drugs were used. Baseline muscle symptom intensity was mean 4.6 (SD 2.5) cm. No changes in consumption of analgesics or non-steroidal anti-inflammatory drugs were reported during the trial period. No patients used concomitant treatment with drugs that interact strongly with atorvastatin, coenzyme Q10 or other non-prescription drugs or supplements.

Outcomes

Atorvastatin did not affect the intensity of muscle symptoms (*Figure 2*). In 17% (9.9% to 27%) $n = 12$ patients, more muscle symptoms were reported on placebo than atorvastatin, with mean VAS difference: -3.2 (95% CI -4.3 to -2.2). In 55% (43% to 66%) $n = 39$ patients, no differences in muscle symptom intensity between atorvastatin and placebo was reported, with mean VAS difference of 0.07 (95% CI: -0.14 to 0.28). In 28% (19% to 40%) $n = 20$ patients, more muscle symptoms were reported on atorvastatin than placebo (i.e. confirmed SAMS), with mean VAS difference: 2.9 (95% CI: 2.1 to 3.6).

Table 1 Baseline characteristics of participants (full analysis set) according to treatment sequence

Characteristic	Atorvastatin → placebo, *N* = 36, (50.7%)	Placebo → atorvastatin, *N* = 35 (49.3%)	Total, *N* = 71
Demographics			
Age (years), mean, (SD)	63.8 (7.8)	63.1 (11.0)	63.5 (9.5)
Female, *N* (%)	12 (33.3)	11 (31.4)	23 (32.4)
Low education,[a] *N* (%)	21 (58.3)	24 (68.6)	45 (63.4)
Non-Caucasian origin, *n* (%)	0 (0)	0 (0)	0 (0)
Index coronary diagnosis			
Myocardial infarction, *N* (%)	30 (83.3)	30 (85.7)	60 (84.5)
Time since last coronary event, months, mean (SD)	25.0 (16.4)	20.4 (10.0)	22.7 (13.7)
Statin treatment and history of intolerance			
Previous atorvastatin discontinuation due to side effects, *N* (%)	13 (36.1)	13 (37.1)	26 (36.6)
Moderate- or low-intensity statin therapy,[b] *n* (%)	19 (52.8)	12 (34.3)	31 (43.7)
No ongoing statin therapy, *N* (%)	5 (13.9)	3 (8.6)	8 (11.3)
Ezetemibe, *N* (%)	10 (27.2)	6 (17.1)	16 (22.5)
Total number of statins used previously, *N* (SD)	1.36 (0.64)	1.31 (0.58)	1.34 (0.61)
Used two different statins previously, *N* (%)	7 (19.4)	7 (20.0)	14 (19.7)
Used three different statins previously, *N* (%)	3 (8.3)	2 (5.7)	5 (7.0)
Cardiovascular risk factors			
Body mass index (kg/m^2), mean, (SD)	29.2 (4.1)	27.3 (4.4)	28.2 (4.4)
Diabetes, *N* (%)	1 (2.8)	4 (11.4)	5 (7.0)
Current smoking, *N* (%)	4 (11.1)	5 (14.3)	9 (13.0)
Low-physical activity,[c] *N* (%)	17 (47.2)	16 (45.7)	33 (46.5)
Laboratory tests			
Creatinine (μmol/L), mean (SD)	80.2 (13.1)	85.1 (33.7)	82.6 (25.5)
Estimated GFR (mL/min/1.73m^2), mean (SD)	79.9 (12.0)	77.5 (16.5)	78.7 (14.3)
Low-density lipoprotein cholesterol (mmol/L), mean (SD)	2.50 (1.19)	2.29 (0.85)	2.40 (1.03)
Creatine kinase (U/L), mean (SD)	136 (99)	146 (94)	141 (96)
Lactate dehydrogenase (mmol/L), mean (SD)	175.4 (28.1)	180.1 (34.7)	177.8 (33.4)
Alanine aminotransferase (U/L), mean (SD)	34.8 (16.5)	35.5 (23.8)	35.1 (20.3)
High-sensitivity C-reactive protein (mg/L), mean (SD)	3.62 (8.08)	2.39 (0.85)	3.01 (6.03)
Comorbidities			
>1 previous coronary event, *N* (%)	10 (27.8)	16 (45.7)	26 (36.6)
Heart failure, *N* (%)	8 (22.2)	6 (17.1)	12 (16.9)
Stroke/transitory ischaemic attack, *N* (%)	2 (5.6)	4 (11.4)	6 (8.5)
Rheumatic or inflammatory disease, *N* (%)	1 (2.8)	0 (0)	1 (1.4)
Arthrosis, *N* (%)	15 (41.7)	10 (32.3)	25 (37.3)
Hypo- or hyperthyroidism, *N* (%)	2 (5.6)	1 (2.9)	3 (4.2)
Anxiety or depression (diagnosis), *N* (%)	6 (16.7)	3 (8.6)	9 (12.7)
Concomitant medication used regularly			
Total number of concomitant drugs, *mean* (SD)	5.3 (2.3)	5.5 (1.9)	5.4 (2.1)
NSAIDs or analgesics, *N* (%)	7 (19.4)	5 (14.3)	12 (16.9)

BMI, body mass index; GFR, glomerular filtration rate; *N*, number; NSAIDS, non-steroidal anti-inflammatory drugs; SD, standard deviation.
[a]Low education was defined by completion of primary and secondary school only.
[b]High-intensity statin therapy means drug regimens that are known to lower low-density lipoprotein cholesterol on average by ∼50% (i.e. ≥40 mg atorvastatin/day or ≥20 mg rosuvastatin/day). All the other drug regimens were considered as low- or moderate-intensity statin treatment.
[c]Physical activity <30 min of moderate intensity two to three times weekly.

Irrespective of the treatment sequence, patients reported similar (mean VAS difference 0.28, 95% CI: -0.28 to 0.83) muscle symptom intensities in the two treatment periods. Two patients, both with confirmed SAMS, experienced intolerable muscle symptoms at Week 4 and 5, leading to discontinuation of treatment.

In a *post hoc* analysis, the distribution of patients to the three groups: more muscle symptoms on placebo ($n = 12$), no difference between atorvastatin and placebo ($n = 39$), and more muscle symptoms on atorvastatin ($n = 20$) was not statistically different from 25%/50%/25% ($P = 0.29$; the Pearson χ^2 test for multinomial probabilities).

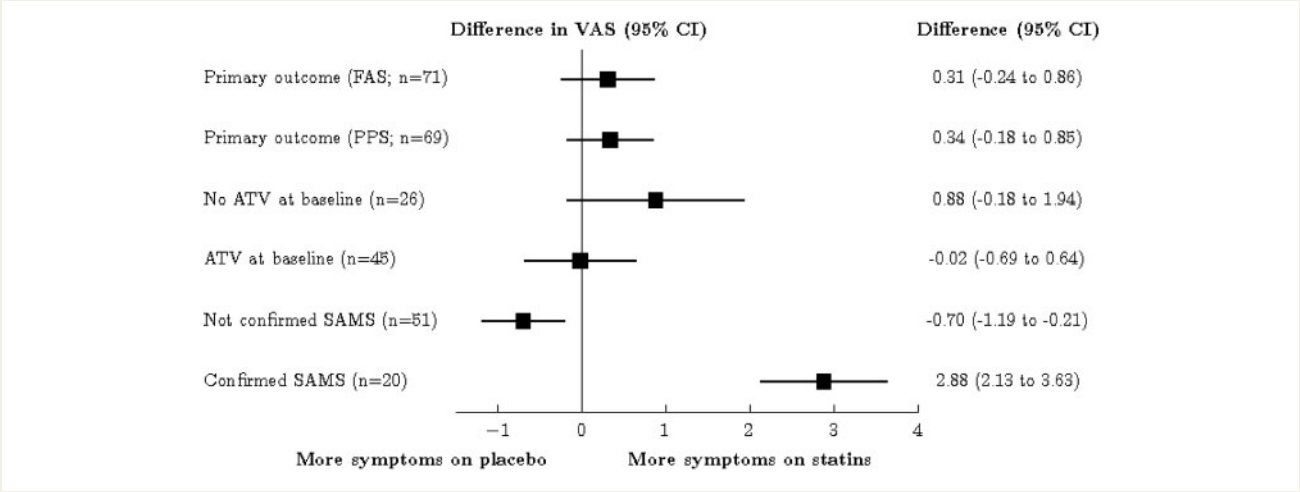

Figure 2 Effect of atorvastatin on muscle symptom intensity in coronary heart disease patients with subjective statin-associated muscle symptoms. ATV, atorvastatin; FAS, full analysis set; PPS, per-protocol set; SAMS, statin-associated muscle symptoms; VAS, visual-analogue scale.

Moreover, when the middle category was excluded, the proportion of patients with more muscle symptoms on atorvastatin was not statistically significantly different from 50% ($P = 0.16$; score test for a single probability). This indicates that the observed distribution of patients to these three groups could be due to chance.

Levels of atorvastatin and/or metabolites in blood plasma did not correlate to the difference between atorvastatin and placebo in muscle symptom intensity among patients with confirmed SAMS (*Table 2*). The individual metabolites and/or sums of metabolites did not discriminate patients with confirmed SAMS from non-SAMS (see Supplementary material online, *Appendix Table S1*). All over, the distributions of metabolite plasma concentrations were comparable between the confirmed SAMS, non-SAMS, and control group patients.

Exploratory comparisons revealed no differences in relevant clinical or pharmacogenetic characteristics between participants with confirmed SAMS and non-SAMS and between the intervention group and the control group without muscle symptoms (*Table 3*). Sixteen out of 19 (84%) patients with self-perceived SAMS on ≥2 statins at study start were classified as non-SAMS.

Table 2 Correlations between the difference in mean muscle symptom intensity and levels of atorvastatin and metabolites among participants with confirmed statin-associated muscle symptoms ($n = 20$)

Drug exposure variable	Spearman's rho (95% CI)
Trough (C0) concentration in nM	
Atorvastatin acid	0.07 (-0.39 to 0.50)
2-OH atorvastatin acid	0.38 (-0.09 to 0.71)
4-OH atorvastatin acid	0.40 (-0.07 to 0.73)
Sum acids	0.31 (-0.16 to 0.67)
Atorvastatin lactone	-0.11 (-0.53 to 0.35)
2-OH atorvastatin lactone	0.27 (-0.20 to 0.64)
4-OH atorvastatin lactone	0.36 (-0.12 to 0.70)
Sum lactones	0.26 (-0.22 to 0.63)
Sum acids and lactones	0.29 (-0.19 to 0.65)
Atorvastatin acylglucuronide	0.11 (-0.35 to 0.53)
Peak (C2) concentration in nM	
Atorvastatin acid	0.07 (-0.38 to 0.50)
2-OH atorvastatin acid	-0.01 (-0.45 to 0.44)
4-OH atorvastatin acid	0.30 (-0.18 to 0.66)
Sum acids	0.10 (-0.36 to 0.52)
Atorvastatin lactone	-0.04 (-0.47 to 0.41)
2-OH atorvastatin lactone	-0.19 (-0.58 to 0.28)
4-OH atorvastatin lactone	0.08 (-0.38 to 0.50)
Sum lactones	-0.17 (-0.57 to 0.29)
Sum acids and lactones	0.01 (-0.43 to 0.45)
Atorvastatin acylglucuronide	-0.08 (-0.50 to 0.38)

C, concentration; CI, confidence interval.

Adverse events

One patient died, most likely due to a primary arrhythmia, and one patient was revascularized due to new-onset angina. Emergency unblinding revealed that both patients received atorvastatin at the time of the adverse event. One patient was un-blinded due to an elevation of alanine aminotransferase >10× upper normal limit at the end of the atorvastatin treatment period, which resolved rapidly when atorvastatin was discontinued. The atorvastatin and metabolites levels in this patient were within the 95% CIs of the mean concentrations in the non-SAMS patients and the control group.

Discussion

In this randomized, double-blinded crossover trial, atorvastatin did not affect the intensity of muscle symptoms among patients with CHD and self-perceived SAMS. The proportion classified with confirmed SAMS (28%), according to our pre-specified definition, is not higher than expected by chance as 17% also reported more symptoms on placebo than on atorvastatin. Although truly

Table 3 Characteristics of participants in the crossover trial and the control group

Characteristic	Confirmed SAMS, *N* = 20 (28.1%)	Non-SAMS, *N* = 51 (71.8%)	Control group, *N* = 40
Baseline characteristics			
Women, *N* (%)	7 (35.0)	16 (31.4)	12 (30.0)
Age (years), mean (SD)	64.1 (11.0)	63.2 (8.9)	64.2 (8.6)
Previous atorvastatin discontinuation, *N* (%)	8 (40.0)	28 (54.9)	0 (0)
High-intensity statin at baseline, *N* (%)	12 (60.0)	28 (54.9)	38 (95.0)
Body mass index (kg/m^2), mean (SD)	27.6 (4.1)	28.5 (4.4)	28.3 (4.1)
High physical activity, *N* (%)	12 (60.0)	27 (54.0)	23 (57.5)
Alanine aminotransferase (U/L), mean (SD)	30.7 (14.9)	36.9 (21.9)	41.6 (23.4)
Creatinine (μmol/L), mean (SD)	84.7 (40.6)	81.9 (16.5)	82.3 (35.6)
Estimated GFR (mL/min/1.73m^2), mean (SD)	78.7 (18.2)	78.5 (12.8)	78.6 (17.4)
Total number of concomitant drugs, *mean* (SD)	5.9 (2.5)	5.2 (1.9)	5.3 (1.6)
Regular use of analgesics, *N* (%)	4 (20.0)	11 (21.6)	3 (7.5)
CYP3A4 *1/*1, *N* (%)	17 (85.0)	47 (92.2)	37 (92.5)
CYP3A4 *1/*22, *N* (%)	3 (15.0)	4 (7.8)	3 (7.5)
CYP3A4 *22/*22, *N* (%)	0 (0)	0 (0)	0 (0)
CYP3A5 *1/*1, *N* (%)	0 (0)	0 (0)	0 (0)
CYP3A5 *1/*3, *N* (%)	3 (15.0)	6 (11.8)	7 (17.5)
CYP3A5 *3/*3, *N* (%)	17 (85.0)	45 (88.2)	33 (82.5)
SLCO1B1 *1/*1, *N* (%)	17 (85.0)	37 (72.6)	26 (65.0)
SLCO1B1 *1/*5, *N* (%)	3 (15.0)	14 (27.5)	13 (32.5)
SLCO1B1 *5/*5, *N* (%)	0 (0)	0 (0)	1 (2.5)
Characteristics during the treatment period on atorvastatin			
Alanine aminotransferase (U/L), mean (SD)	29.9 (14.4)[a]	33.5 (17.1)	45.0 (59.7)
Creatine kinase (U/L), mean (SD)	102 (41.1)	152 (83.6)	128 (77.6)
Lactate dehydrogenase (mmol/L), mean (SD)	165 (35.0)	180 (37.2)	181 (28.3)

C, concentration; CI, confidence interval; SAMS, statin-associated muscle symptom; SD, standard deviation.
[a]One patient with an adverse reaction (i.e. elevation of alanine aminotransferase >10× upper normal limit) at the end of the atorvastatin treatment period was excluded from this analysis.

statin-dependent muscle symptoms are not excluded in a minority of patients, they are likely to be rare compared with the reported prevalence of 10–25%.

MUSE is the first randomized crossover trial designed and powered to determine the effect of statin treatment on muscle symptoms in patients with self-perceived SAMS. The consecutively screened population from routine clinical practice is important for the generalizability of the results. Our prevalence estimate of self-perceived SAMS (10%) was the same as that reported in the large PRIMO survey, exploring the association between high-dose statins and self-reported muscle symptoms in general practice.[9] However, the inherent biases of PRIMO and other observational studies[7,8] limit their ability to evaluate causality.[3]

STOMP[34] was a randomized, blinded trial designed to assess the effect of atorvastatin 80 mg/day on several muscle-related measures in healthy individuals. They reported a small excess of myalgia in statin-treated individuals as compared with placebo (19 vs. 10, $P = 0.05$). The effect of statins on muscle symptoms in the individual patient could, however, not be determined as STOMP was not a crossover study. A two-phase randomized trial (GAUSS-3),[16] enrolling patients with poorly controlled low-density lipoprotein cholesterol levels and history of intolerance to two or more statins, applied a crossover procedure to identify eligible patients for testing the effect of two different non-statin therapies. They found that 43% had muscle symptoms on atorvastatin 20 mg and not on placebo whereas 27% had muscle symptoms on placebo and not on atorvastatin,[16] yielding the same risk ratio of 1.5 as found in our study. However, the results of the crossover phase of GAUSS-3 should be considered suggestive as they were subject to an exploratory analysis without predefined methods in the statistical analysis plan. A similar two-phase crossover trial, investigating the effect of coenzyme Q10 for the treatment of SAMS, found that 36% had muscle symptoms on simvastatin 20 mg and not on placebo as compared with 29% on placebo and not on simvastatin.[17] In contrast to the present study, the Q10 and GAUSS-3 trials were not specifically designed to determine the effect of statins on muscle symptom intensity and potentially eligible participants were not consecutively screened. There was a somewhat lower proportion with statin-dependent muscle symptoms in MUSE (28%) as compared with these trials (42% and 36%). Differences in how muscle symptoms were measured as well as

patient selection of may explain these differences. Eighty-one per cent reported intolerance to three or more statins prior to study start in GAUSS-3, whereas only 7% ($n = 5$) in our study had tried that many statins. Interestingly, all these patients were classified as non-SAMS in our study. Indeed, 16 out of 19 patients with a history of intolerance to ≥2 statins were also classified as non-SAMS. Importantly, atorvastatin did not affect the intensity of muscle symptoms in our primary analysis, and the proportion with confirmed SAMS according to our pre-specified and validated definition was not significantly higher than expected by chance alone. Accordingly, our exploratory analyses revealed no differences in clinical characteristics between patients categorized with confirmed SAMS and non-SAMS. If a subgroup of the patients with statin-dependent muscle symptoms actually exists, the prevalence is likely to be low. The proportion with more symptoms on placebo than atorvastatin may be explained by fluctuations in statin-independent muscle symptoms, alternatively the nocebo effect.[35] Interestingly, a small non-randomized study ($n = 8$) with several crossovers indicates no differences in muscle symptoms between statin and placebo in patients with SAMS.[36]

In this first study, testing also the metabolites of a statin as mediators of SAMS. There was no correlation between muscle symptom intensity and systemic exposure to atorvastatin and its main metabolites. Several *in vitro* studies have reported that lactone metabolites of statins induce toxic effects in muscle.[18] In a previous study, patients were classified with SAMS according to open statin re/de-challenge and their blood levels of atorvastatin metabolites were compared with healthy individuals, both groups using low-dose (10 mg/day) atorvastatin.[21] The blood levels of atorvastatin lactone and 4-OH atorvastatin acid were higher in the SAMS patients.[21] Our placebo-controlled trial demonstrates that the intensity of muscle symptoms is not related to the concentrations of atorvastatin or any of its main metabolites in blood. Moreover, the frequency of sequence variants in CYP3A4/5 and SLCO1B1 was not different between the participants in the randomized trial and the control group without any history of muscle complaints. Potentially, the toxic effects of atorvastatin that occur in the muscle tissue are not adequately reflected by blood plasma concentrations of the drug and metabolites. *In vitro* experiments indicate that influx and efflux transporter proteins are determinants of the local exposure to statin metabolites in skeletal muscle tissue.[37] Consequently, it can be hypothesized that the levels of statin metabolites in muscle tissue are not directly correlated to the exposure in blood. Future studies should obtain muscle biopsies to elucidate further the relationship between muscle symptoms and atorvastatin metabolites and other biomarkers in patients with confirmed SAMS.

Self-perceived SAMS is common (10%) but our conservative estimate of statin-dependent muscle symptoms (<3%) is in line with the estimates of side effects reported in landmark randomized statin trials.[38] Thus, in the clinical perspective, atorvastatin is well tolerated in most patients. A detailed clinical interview elucidating other causes of muscle complaints in these patients appears crucial. Our results may be useful for an informed discussion with patients regarding the likelihood of whether their muscle complaints may be caused by the statin or not. Finally, continuously lowered cholesterol treatment targets together with the emergence of new and expensive lipid-lowering drugs[39] emphasizes the need for optimized use of the cost-effective statins.

Strengths and limitations

The study design enables us to confirm whether the participants' muscle symptoms were truly related to the statin or not, thus addressing the major criticisms of previous SAMS studies.[2] Other strengths include a very low dropout rate, high data quality, and superior adherence to the allocated treatment measured with robust methods.

In all, 802 of 982 patients (82% response rate) responded to the invitation letter and phone calls. Since some of the non-responders may also have experienced SAMS, the prevalence of subjective SAMS in this population could have been slightly higher than the reported estimate. Although observational studies among patients with subjective SAMS[9,27] indicate that the duration of treatment periods should be sufficient for SAMS to appear and disappear in most patients, some participants with perceived changes in muscle symptom intensity after the 8 weeks treatment period may have been incorrectly classified. Even though the time to first noted recovery in muscle symptoms following statin discontinuation was median 2 weeks in a case study of 354 patients with self-perceived SAMS, the time to complete recovery was median 4 weeks.[27] Although it is biologically unlikely that muscle complaints persist for more than 5 weeks after statin discontinuation, the possibility of carryover effects of muscle symptoms in participants with symptoms lasting longer cannot be entirely excluded. To minimize the risk of carryover effects, the outcomes were evaluated only during the last 3 weeks of each test-period of 7 weeks. In addition, the washout periods of 1 week ensured complete pharmacologic clearance of ongoing statin treatment used prior to study start or in the preceding placebo treatment period. Muscle symptoms were registered in a diary that was available for the participants throughout the trial, which could possibly have affected the participants' responses in that previously registered VAS scores may have been used as assessment of symptoms in a subsequent week. The diary was chosen to also allow for participation of elderly patients without access to the internet or mobile phones. Eighty-eight per cent of the present participants who had previously tried 2 or 3 different statins prior to inclusion were classified as non-SAMS by our blinded crossover procedure. Accordingly, reporting side effects after two different statins, or more, does not appear to be a valid marker of true SAMS. Thus, identification of SAMS by the suggested open de-challenge/re-challenge tests[5] remains to be validated and their sensitivity and specificity determined. Such tests are also rarely performed in clinical practice, as none had been performed among the patients screened for the present study. Future studies may establish the validity of both clinical algorithms and screening questionnaires in predicting statin-dependent muscle side effects. Even though atorvastatin is used by the majority of CHD patients, the study results are not outright representative of other statin classes. Moreover, there are regional differences in the distribution of cardiovascular risk factors across Europe, and possibly also in the prevalence and characteristics of the

population with SAMS. Finally, all study participants were Caucasian and the study results should be interpreted accordingly.

Conclusions

Double-blinded re-challenge with high-intensity atorvastatin did not affect the intensity of muscle symptoms in patients with CHD and self-perceived SAMS during previous atorvastatin therapy. There was no relationship between muscle symptoms and the systemic exposure to atorvastatin and/or its metabolites. The findings encourage an informed discussion to elucidate other causes of muscle complaints and continued statin use.

Supplementary material

Supplementary material is available at *European Heart Journal – Cardiovascular Pharmacotherapy* online.

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Conflict of interest: O.K. reports having received modest lecture fees from Astra Zeneca, Novartis, and Bayer, outside the submitted work. J.M. reports having received modest lecture fees from Sanofi, Amgen, and Bayer, outside the submitted work. E.G. reports having received modest lecture fees from BMS, Pfizer, Boeringer Ingelheim, and Sanofi, outside the submitted work. L.G. reports having received modest lecture fees from Astra Zeneca, Novo, Amgen, and Sanofi, outside the submitted work. No other disclosures were reported.

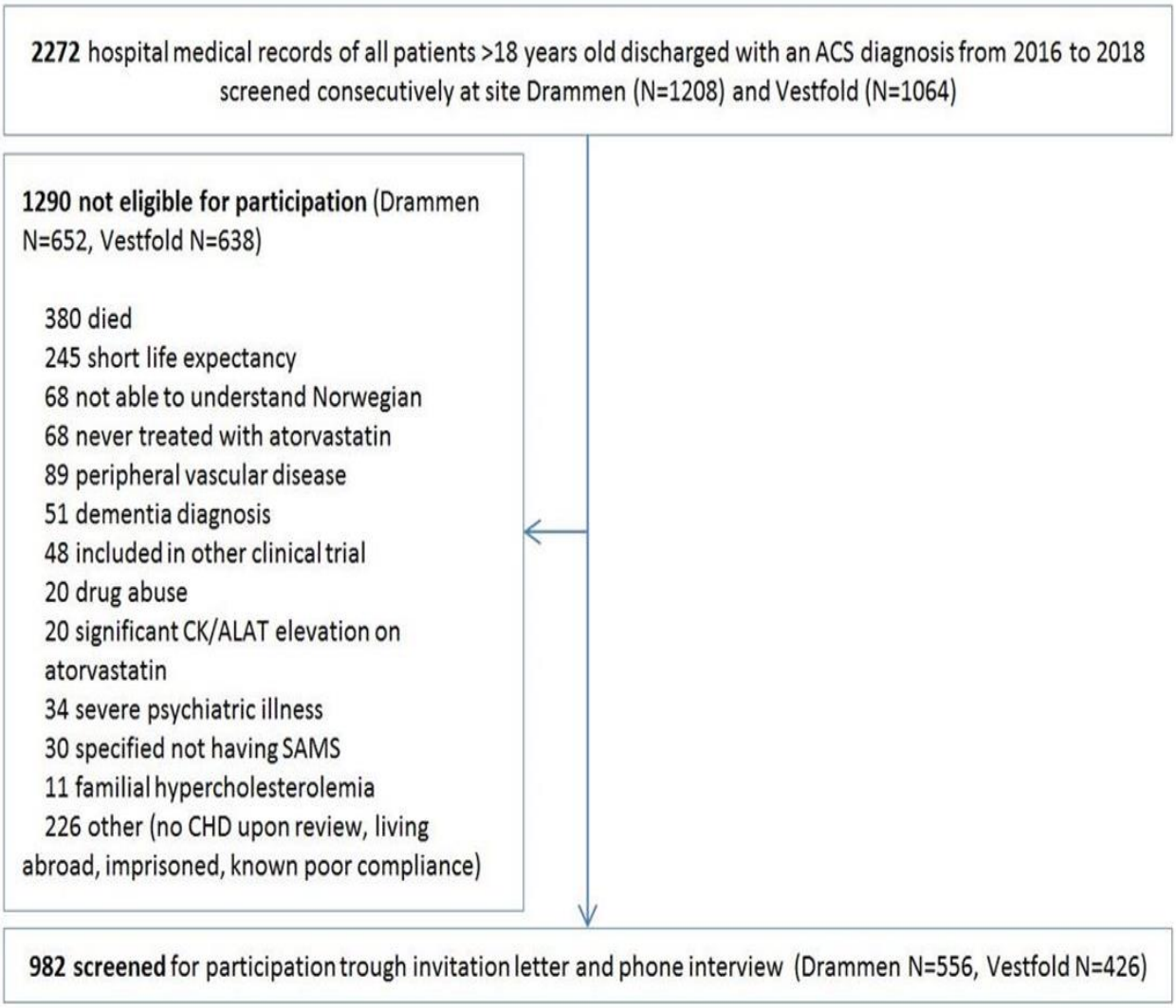
References

1. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, Graham IM, Halliday A, Landmesser U, Mihaylova B, Pedersen TR, Riccardi G, Richter DJ, Sabatine MS, Taskinen M-R, Tokgozoglu L, Wiklund O, ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J* 2020;**41**:111–188.
2. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R, Cholesterol Treatment Trialists Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010;**376**:1670–1681.
3. Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, Blumenthal R, Danesh J, Smith GD, DeMets D, Evans S, Law M, MacMahon S, Martin S, Neal B, Poulter N, Preiss D, Ridker P, Roberts I, Rodgers A, Sandercock P, Schulz K, Sever P, Simes J, Smeeth L, Wald N, Yusuf S, Peto R. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016;**388**:2532–2561.
4. Vinogradova Y, Coupland C, Brindle P, Hippisley-Cox J. Discontinuation and restarting in patients on statin treatment: prospective open cohort study using a primary care database. *BMJ* 2016;**353**:i3305.
5. Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, Roden M, Stein E, Tokgözoğlu L, Nordestgaard BG, Bruckert E, De Backer G, Krauss RM, Laufs U, Santos RD, Hegele RA, Hovingh GK, Leiter LA, Mach F, März W, Newman CB, Wiklund O, Jacobson TA, Catapano AL, Chapman MJ, Ginsberg HN, European Atherosclerosis Society Consensus Panel. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. *Eur Heart J* 2015;**36**:1012–1022.
6. De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. *Br J Clin Pharmacol* 2014;**78**:684–698.
7. Buettner C, Rippberger MJ, Smith JK, Leveille SG, Davis RB, Mittleman MA. Statin use and musculoskeletal pain among adults with and without arthritis. *Am J Med* 2012;**125**:176–182.
8. Mansi I, Frei CR, Pugh MJ, Makris U, Mortensen EM. Statins and musculoskeletal conditions, arthropathies, and injuries. *JAMA Intern Med* 2013;**173**:1–10.
9. Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients-the PRIMO study. *Cardiovasc Drugs Ther* 2005;**19**:403–414.
10. Cohen JD, Brinton EA, Ito MK, Jacobson TA. Understanding Statin Use in America and Gaps in Patient Education (USAGE): an internet-based survey of 10,138 current and former statin users. *J Clin Lipidol* 2012;**6**:208–215.
11. Kashani A, Phillips CO, Foody JM, Wang Y, Mangalmurti S, Ko DT, Krumholz HM. Risks associated with statin therapy: a systematic overview of randomized clinical trials. *Circulation* 2006;**114**:2788–2797.
12. Finegold JA, Manisty CH, Goldacre B, Barron AJ, Francis DP. What proportion of symptomatic side effects in patients taking statins are genuinely caused by the drug? Systematic review of randomized placebo-controlled trials to aid individual patient choice. *Eur J Prev Cardiol* 2014;**21**:464–474.
13. Desai CS, Martin SS, Blumenthal RS. Non-cardiovascular effects associated with statins. *BMJ* 2014;**349**:g3743.
14. Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J, Gupta M, Hegele RA, Ng D, Pearson GJ, Pope J, Tashakkor AY. Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Consensus Working Group Update (2016). *Can J Cardiol* 2016;**32**:S35–S65.
15. Joy TR, Monjed A, Zou GY, Hegele RA, McDonald CG, Mahon JL. N-of-1 (single-patient) trials for statin-related myalgia. *Ann Intern Med* 2014;**160**:301–310.
16. Nissen SE, Stroes E, Dent-Acosta RE, Rosenson RS, Lehman SJ, Sattar N, Preiss D, Bruckert E, Ceska R, Lepor N, Ballantyne CM, Gouni-Berthold I, Elliott M, Brennan DM, Wasserman SM, Somaratne R, Scott R, Stein EA; Gauss-3 Investigators. Efficacy and tolerability of evolocumab vs ezetimibe in patients with muscle-related statin intolerance: the GAUSS-3 randomized clinical trial. *JAMA* 2016;**315**:1580–1590.
17. Taylor BA, Lorson L, White CM, Thompson PD. A randomized trial of coenzyme Q10 in patients with confirmed statin myopathy. *Atherosclerosis* 2015;**238**:329–335.
18. Ward NC, Watts GF, Eckel RH. Statin toxicity. *Circ Res* 2019;**124**:328–350.
19. Rosenson RS, Baker SK, Jacobson TA, Kopecky SL, Parker BA. The National Lipid Association's Muscle Safety Expert P: an assessment by the Statin Muscle Safety Task Force: 2014 update. *J Clin Lipidol* 2014;**8**:S58–S71.
20. Moßhammer D, Schaeffeler E, Schwab M, Mörike K. Mechanisms and assessment of statin-related muscular adverse effects. *Br J Clin Pharmacol* 2014;**78**:454–466.
21. Hermann M, Bogsrud MP, Molden E, Asberg A, Mohebi BU, Ose L, Retterstol K. Exposure of atorvastatin is unchanged but lactone and acid metabolites are increased several-fold in patients with atorvastatin-induced myopathy. *Clin Pharmacol Ther* 2006;**79**:532–539.
22. Skottheim IB, Gedde-Dahl A, Hejazifar S, Hoel K, Åsberg A. Statin induced myotoxicity: the lactone forms are more potent than the acid forms in human skeletal muscle cells in vitro. *Eur J Pharm Sci* 2008;**33**:317–325.
23. Schirris TJ, Renkema GH, Ritschel T, Voermans NC, Bilos A, van Engelen BG, Brandt U, Koopman WJ, Beyrath JD, Rodenburg RJ, Willems PH, Smeitink JA, Russel FG. Statin-induced myopathy is associated with mitochondrial complex III inhibition. *Cell Metab* 2015;**22**:399–407.
24. Munkhaugen J, Vethe NT, Fagerland MW, Dammen T, Perk J, Gjertsen E, Otterstad JE, Gullestad L, Bergan S, Husebye E. Statin-associated muscle

symptoms in coronary patients: design of a randomized study. *Scand Cardiovasc J* 2019;**53**:162–168.
25. Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 statement: extension to randomised crossover trials. *BMJ* 2019;**366**:l4378.
26. Statistics Norway (date of origination: 25 may 2016) https://www.ssb.no/statistikkbanken (14 June 2020), http://cvdnor.b.uib.no/files/2013/08/CVDNOR-Data-and-Quality-Report.pdf (14 June 2020).
27. Cham S, Evans MA, Denenberg JO, Golomb BA. Statin-associated muscle-related adverse effects: a case series of 354 patients. *Pharmacotherapy* 2010;**30**:541–553.
28. Garcia MJ, Reinoso RF, Sanchez Navarro A, Prous JR. Clinical pharmacokinetics of statins. *Methods Find Exp Clin Pharmacol* 2003;**25**:455–481.
29. Vethe NT, Munkhaugen J, Andersen AM, Husebye E, Bergan S. A method for direct monitoring of atorvastatin adherence in cardiovascular disease prevention: quantification of the total exposure to parent drug and major metabolites using 2-channel chromatography and tandem mass spectrometry. *Ther Drug Monit* 2019;**41**:19–28.
30. Kristiansen O, Vethe NT, Fagerland MW, Bergan S, Munkhaugen J, Husebye E. A novel direct method to determine adherence to atorvastatin therapy in patients with coronary heart disease. *Br J Clin Pharmacol* 2019;**85**:2878.
31. Gallagher EJ, Liebman M, Bijur PE. Prospective validation of clinically important changes in pain severity measured on a visual analog scale. *Ann Emerg Med* 2001;**38**:633–638.
32. Fagerland MW, Lydersen S, Laake P. *Statistical Analysis of Contingency Tables, Chapter 2*. Boca Raton, FL: Chapman & Hall/CRC; 2017.
33. Fagerland MW, Lydersen S, Laake P. *Statistical Analysis of Contingency Tables, Chapter 7*. Boca Raton, FL: Chapman & Hall/CRC; 2017.
34. Parker BA, Capizzi JA, Grimaldi AS, Clarkson PM, Cole SM, Keadle J, Chipkin S, Pescatello LS, Simpson K, White CM, Thompson PD. Effect of statins on skeletal muscle function. *Circulation* 2013;**127**:96–103.
35. Tobert JA, Newman CB. The nocebo effect in the context of statin intolerance. *J Clin Lipidol* 2016;**10**:739–747.
36. Herrett E, Williamson E, Beaumont D, Prowse D, Youssouf N, Brack K, Armitage J, Goldacre B, MacDonald T, Staa TV, Roberts I, Shakur-Still H, Smeeth L. Study protocol for statin web-based investigation of side effects (StatinWISE): a series of randomised controlled N-of-1 trials comparing atorvastatin and placebo in UK primary care. *BMJ Open* 2017;**7**:e016604.
37. Knauer MJ, Urquhart BL, Meyer zu Schwabedissen HE, Schwarz UI, Lemke CJ, Leake BF, Kim RB, Tirona RG. Human skeletal muscle drug transporters determine local exposure and toxicity of statins. *Circ Res* 2010;**106**:297–306.
38. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;**344**:1383–1389.
39. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Goodman SG, Hanotin C, Harrington RA, Jukema JW, Lecorps G, Mahaffey KW, Moryusef A, Pordy R, Quintero K, Roe MT, Sasiela WJ, Tamby JF, Tricoci P, White HD, Zeiher AM, ODYSSEY OUTCOMES Committees and Investigators. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med* 2018;**379**:2097–2107.

SUPPLEMENTARY MATERIAL PAPER 3

Paper 3 - Supplementary Figure 1 – Causes of exclusions during consecutive screening of hospital discharge lists prior to telephone interviews.



Paper 3 - Supplementary Table 1 – Diagnostic properties of atorvastatin and metabolites for the prediction of confirmed statin-associated muscle symptoms (*n*=20/71 (28%)) among patients with subjective statin associated muscle symptoms.

Analyte	Discriminator (nM)	Sensitivity	Specificity	AUC (95% CI)
Through concentration in nM				
Atorvastatin acid	0.99	50%	71%	0.51 (0.35 to 0.68)
2-OH atorvastatin acid	1.17	50%	57%	0.49 (0.31 to 0.66)
4-OH atorvastatin acid	0.59	50%	65%	0.50 (0.32 to 0.67)
Sum acids	2.52	50%	57%	0.51 (0.34 to 0.69)
Atorvastatin lactone	1.20	65%	43%	0.46 (0.31 to 0.61)
2-OH atorvastatin lactone	2.67	50%	71%	0.52 (0.34 to 0.69)
4-OH atorvastatin lactone	0.92	50%	53%	0.48 (0.32 to 0.65)
Sum lactones	5.11	50%	59%	0.50 (0.34 to 0.67)
Sum acids and lactones	7.77	50%	61%	0.51 (0.34 to 0.68)
Atorvastatin acylglucuronide	0.034	50%	53%	0.48 (0.33 to 0.63)
Peak exposure in nM				
Atorvastatin acid	22.1	60%	47%	0.50 (0.34 to 0.65)
2-OH atorvastatin acid	11.0	75%	25%	0.42 (0.28 to 0.56)
4-OH atorvastatin acid	1.84	50%	53%	0.48 (0.34 to 0.63)
Sum acids	42.5	55%	49%	0.46 (0.32 to 0.61)
Atorvastatin lactone	30.2	45%	67%	0.53 (0.38 to 0.69)
2-OH atorvastatin lactone	19.9	75%	35%	0.52 (0.37 to 0.68)
4-OH atorvastatin lactone	2.77	70%	29%	0.45 (0.31 to 0.60)
Sum lactones	40.1	75%	33%	0.51 (0.35 to 0.66)
Sum acids and lactones	61.9	85%	27%	0.49 (0.34 to 0.64)
Atorvastatin acylglucuronide	2.11	50%	63%	0.56 (0.40 to 0.72)

Abbreviations: AUC: Area under the receiver operating characteristic curve.