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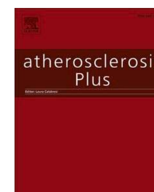

The atorvastatin metabolite pattern in muscle tissue and blood plasma is associated with statin muscle side effects in patients with coronary heart disease; An exploratory case-control study

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ABSTRACT

Background and aims: Statin-associated muscle symptoms (SAMS) is a prevalent cause of statin discontinuation. It is challenging and time-consuming for clinicians to assess whether symptoms are caused by the statin or not, and diagnostic biomarkers are requested. Atorvastatin metabolites have been associated with SAMS. We aimed to compare atorvastatin pharmacokinetics between coronary heart disease (CHD) patients with and without clinically statin intolerance and statin-dependent histopathological alterations in muscle tissue. Secondarily we aimed to assess genetic variants relevant for the observed pharmacokinetic variables.
Methods: Twenty-eight patients with CHD and subjective SAMS were included in the exploratory MUSE biomarker study in 2020. Participants received atorvastatin 40 mg/day for seven weeks followed by no statins for eight weeks. Muscle biopsies and blood were collected at the end of each period. Four patients were categorized as clinically intolerant to ≥3 statins prior to study start whereas four patients had signs of muscle cell damage during treatment.
Results: We found significantly lower levels of atorvastatin acids, and higher lactone/acid ratios in the statin intolerant, both in muscle and plasma. With optimal cut-off, the combination of 2-OH-atorvastatin acid and the 2-OH-atorvastatin lactone/acid ratio provided sensitivity, specificity, and predictive values of 100 %. Patients with variants in *UGT1A1* and *UGT1A3* had higher lactone metabolite levels than those with wild type, both in muscle and plasma.
Conclusion: Atorvastatin metabolites appear promising as biomarkers for the identification of clinical statin intolerance in patients with self-perceived SAMS, but the findings have to be confirmed in larger studies.

1. Introduction

Cholesterol-lowering treatment with a statin is strongly recommended in the prevention of atherosclerotic cardiovascular disease (ASCVD) [1]. Early discontinuation of statin therapy elevates the risk of myocardial infarction and ASCVD mortality [2–4]. One of the most frequent reasons for statin discontinuation is statin-associated muscle symptoms (SAMS), which may include myalgia, tenderness, stiffness, cramps and weakness [5]. Such symptoms are rarely accompanied by clinical significant increase of creatine kinase in blood [6]. Recent studies have shown that the nocebo effect applies in many patients with SAMS, and it is estimated that up to 90 % of self-perceived muscle side

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effects do not depend on the statin [5,7,8]. To differentiate between statin-dependent muscle side effects and muscle symptoms caused by other factors, an objective measure is needed, that is both reliable and easily accessible.

Based on *in vitro* studies, atorvastatin lactone metabolites have demonstrated the potential to induce toxic effects in skeletal muscle cells, possibly by impairing the mitochondrial respiratory chain [7,9]. However, results from clinical studies are inconsistent about how atorvastatin pharmacokinetic variables relate to muscle symptoms. In patients with SAMS, higher absolute levels of atorvastatin metabolites [10], higher lactone/acid metabolite ratios [11], and no association [12] have been reported for blood plasma analyses. Although the *SLCO1B1* c.521T > C (rs4149056) genetic variant causes elevated systemic levels of atorvastatin metabolites [13], the association with SAMS in the context of atorvastatin therapy is inconsistent [12,14]. Also, the *SLCO2B1* c.935G > A (rs12422149) variant has been associated with SAMS [15]. Lactone metabolites of atorvastatin are formed in a two-step process with acyl glucuronide metabolites as intermediates. In this biosynthesis, UDP-glucuronosyltransferase 1A3 (UGT1A3) is important for the glucuronidation rate [16]. The *UGT1A3*2* haplotype has been associated with increased expression of the enzyme and, thus, increased lactonization of atorvastatin and its metabolites [16,17]. More recently, the *UGT1A1* c.4652C > T (rs887829) variant has been shown to influence the metabolism of atorvastatin, which was evident through higher relative levels of hydroxylated metabolites without direct effect on lactonization [12].

Further pathophysiologic knowledge of patients with SAMS and identification of diagnostic biomarkers have been pointed out as major needs to improve cholesterol management with statins [5].

In 2019, we performed a randomized, double-blinded crossover trial (MUSE RCT) to i. Investigate the effect of atorvastatin 40 mg daily on muscle symptom intensity in coronary heart disease patients with self-perceived SAMS and ii. To determine the relationship between blood levels of atorvastatin metabolites and SAMS according to a prespecified classification of SAMS (i.e., significantly more muscle symptoms during atorvastatin than placebo) [18,19]. Despite a blinded design, the results were apparently influenced by the nocebo effect and fluctuations in muscle symptoms, as 17 % reported more symptoms on placebo than on atorvastatin [18]. Furthermore, only a subgroup of the participants had documented intolerance to ≥3 statins before the study started. These methodological issues may explain the absence of correlation between SAMS and atorvastatin metabolites in our previous studies [18,19]. Accordingly, an evaluation of atorvastatin pharmacokinetics in a population identified based on a stricter and more reliable classification of SAMS is justified [20].

The primary aim of the MUSE biomarker study was to compare atorvastatin pharmacokinetics between coronary heart disease patients with and without clinical statin intolerance and with objective histopathological alterations in muscle tissue due to atorvastatin treatment. Secondarily, we aimed to assess pharmacogenetic variants that may be relevant to the observed pharmacokinetic variables.

2. Patients and methods

2.1. Study design and participants

The present MUscle Side Effects of atorvastatin in coronary patients (MUSE) biomarker study, was a prospective, open intervention study conducted during autumn 2020 at two secondary care hospitals (Drammen Hospital and Vestfold Hospital Trust) in Norway. After one week of washout, all participants received seven weeks of open treatment with atorvastatin 40 mg/day, followed by a period of eight weeks without statin treatment. At the end of each period, we collected blood samples and skeletal muscle biopsies. All patients previously identified as having more symptoms on atorvastatin (n = 20) in the placebo-controlled single crossover MUSE RCT (NCT03874156) were invited to the MUSE biomarker study. In addition, we included 15 randomly selected patients with no differences in muscle symptom intensity between blinded placebo and atorvastatin treatment. The design and method of MUSE RCT is previously described [21]. In brief, the RCT included 71 patients with subjective SAMS during ongoing atorvastatin therapy or previous muscle symptoms that had led to discontinuation of atorvastatin [18]. After one week of washout, the participants received placebo or atorvastatin 40 mg/day, in random order, for seven weeks before a second washout and switching to the other treatment. The patients registered muscle symptom intensity weekly on a visual analog scale (VAS). Mean VAS scores from the last three weeks in each period were used to calculate the difference between the two treatment periods. Higher symptom intensity in one of the periods was predefined as >1 cm and >25 % difference combined. We identified 20 patients (28 %) with more muscle symptoms on atorvastatin than placebo. Furthermore, 39 patients (55 %) did not have different symptom intensity between the treatment periods, and 12 patients (17 %) reported more symptoms on placebo than atorvastatin. The study also included a control group of 40 patients with CHD and no history of muscle complaints associated with statin treatment.

At the end of the trial, 70 out of 71 participants attended an open follow-up study led by cardiologists aiming to tailor and optimize treatment with statins and non-statin lipid-lowering therapy [20]. At the end of the follow-up, mean 13 months later, 64 patients (91 %) tolerated and used statins, and 27 patients used ezetimibe 10 mg daily. Of the 20 patients classified with more symptoms on atorvastatin than placebo, 14 tolerated treatment with another statin, predominantly low-dose rosuvastatin, whereas six patients were categorized as statin intolerant as they did not tolerate ≥3 statins. According to the statin-related myotoxicity phenotype classification, they belonged to group 2 with intolerable myalgia and no or minor increase in creatine kinase [22].

The MUSE RCT and MUSE biomarker study were approved by the Regional Committee for Medical Research Ethics (2018/2302 and 54041) and by the Norwegian Medicines Agency (18/17102-16 and 20/0480-10). EudraCT Number 2019-003959-11. All participants gave a written informed consent to participate, and the study protocol conformed the Declaration of Helsinki.

2.2. Statin intolerance and histopathological alterations in muscle tissue

An overview of participants flow in the MUSE biomarker study is shown in Fig. 1. Patients classified with more symptoms on atorvastatin than placebo in the MUSE RCT and who did not tolerate ≥3 statins in the post-trial follow-up were defined as clinically statin intolerant. In the present biomarker study, histological examination of muscle biopsies on and off atorvastatin was performed with light microscope by an experienced pathologist blinded to statin tolerance status.

2.3. Samples and measurements

We collected venous blood samples and muscle biopsies from the m. quadriceps femoris (caput vastus laterale) 24 h after the atorvastatin dose (T0) at the end of the first treatment period and again at the end of the second period without statin. In addition, we sampled blood at baseline and 1 and 2 h after atorvastatin dose (T1 and T2) at the end of the first period.

The muscle tissue was homogenized for atorvastatin analysis as described previously [19]. Atorvastatin and its main metabolites (atorvastatin acid and lactone, 2-OH-atorvastatin acid and lactone, and 4-OH-atorvastatin acid and lactone) were quantified in muscle tissue and blood plasma with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Waters, Milford, MA) [23]. Atorvastatin acyl glucuronide was estimated in plasma with LC-MS/MS, using atorvastatin acid as a calibrator. The gene variants *SLCO1B1* NM_006446.5: c.521T > C (*5; rs4149056) and *SLCO2B1* NM_007256.5:c.935G > A (rs12422149) were determined by real-time polymerase chain reaction

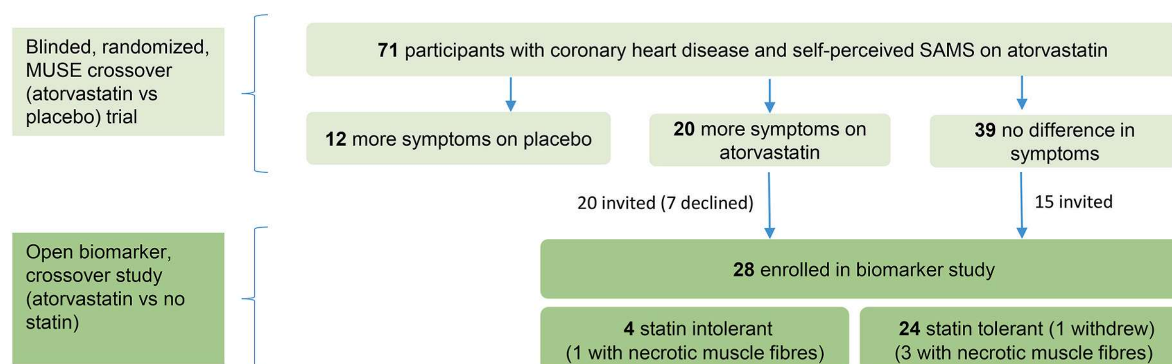

Fig. 1. Participants flow chart. Seventy-one patients with coronary heart disease and self-perceived statin-associated muscle symptoms (SAMS) on atorvastatin treatment had previously been classified with more symptoms on atorvastatin than placebo (n = 20), more symptoms on placebo (n = 12) or no difference in symptoms (n = 39) in the blinded, randomized, MUSE (atorvastatin vs placebo) crossover trial. During follow-up after MUSE RCT, the patients were re-classified as clinically intolerant (not tolerating ≥3 statins, with intolerable muscle symptoms and no or minor increase in creatine kinase) or tolerant to statins. Necrotic muscle fibres were present on atorvastatin and absent off statin in the open crossover study.

analysis (LightCycler 480, Roche Diagnostics, Basel, Switzerland). We determined *UGT1A3* NM_019093.4:c.-758A > G (rs2008584), c.-751T > C (rs1983023), c.808A > G (rs45449995), and *UGT1A1* NG_033238.1:c.4652C > T (rs887829) with custom designed multiplex SNV assays run on the MassArray platform (Agena, San Diego, CA, USA) according to the manufacturer's instructions.

2.4. Variables and statistics

We compared pharmacokinetic variables in muscle and blood between patients with clinical statin intolerance and necrotic muscle fibres, respectively, to those with statin tolerance i.e., absolute levels of atorvastatin and its metabolites, the sum of atorvastatin and its acid metabolites, the sum of atorvastatin lactone metabolites, and lactone/acid ratios of these variables. The pharmacokinetic variables were assessed against the predefined gene variants in *SLCO1B1*, *SLCO2B1*, *UGT1A3*, and *UGT1A1* (only lactone/acid ratios against *SLCO1B1* and *SLCO2B1* since the other pharmacokinetic variables have been assessed against these gene variants previously [19]).

We used non-parametric methods since the small case groups did not allow assumptions about distribution: the Mann-Whitney *U* test for comparing independent, continuous variables between two groups and the Wilcoxon signed rank test for comparison between related samples. Results with 2-tailed p-values <0.05 were considered statistically significant. Due to the exploratory study design, we did not adjust for multiple analyses. The predictive values were calculated with a prevalence of 10 % statin intolerant patient among patients with self-perceived SAMS [20]. Statistical analyses were performed with IBM SPSS version 26 (IBM, Armonk, NY, USA). Figures were made with Prism 9 (GraphPad Software, San Diego, CA, USA) and Powerpoint 2016 (Microsoft Corporation, Redmont, WA, USA).

3. Results

3.1. Participants and descriptive data

We included 28 participants in the MUSE biomarker study (Fig. 1), including 13 out of 20 patients with more muscle symptoms on atorvastatin than placebo and 15 out of 15 patients with no difference in muscle symptoms between the blinded treatment periods in the MUSE RCT. Four patients did not tolerate ≥3 statins during the post-trial follow-up. Necrotic muscle fibres were observed in four patients in the biomarker study, only in the biopsy taken during atorvastatin treatment. One patient with necrotic muscle fibres was clinically statin intolerant. None of the patients had signs of inflammation in the muscle biopsy. The remaining 21 participants were categorized as statin tolerant. One participant in this group was lost to follow-up after the baseline visit, and one participant ingesting the atorvastatin dose a short time before

Table 1
Characteristics of patients at baseline.

Descriptive variable	Self-perceived SAMS > statin tolerant	Self-perceived SAM > statin intolerant	Statin-dependent necrotic muscle fibres
	n = 21	n = 4[a]	n = 4[a]
Age, years	67 (49–80)	61 (38–66)	67 (64–71)
Female, n	7	1	1
Education high, n	8	1	1
Atorvastatin, n	11	0	1
Atorvastatin mg	60 (30–80)	0	20
Other statin[b], n	10	0	2
Ezetimibe, n	6	3	3
Regular medications	6 (2–14)	4 (3–5)	4.5 (3–7)
Muscle symptom intensity, VAS cm	6.6 (0.1–9.8)	2.6 (1.0–5.6)	3.0 (0.2–5.9)
Pain medication, regularly, n	4	0	0
Creatine kinase, U/L	147 (30–490)	103 (66–139)	113 (74–196)
GFR estimated, mL/min/1.73 m^2	83 (38–90)	90 (74–90)	83 (60–90)
HbA1c, mmol/mol	41 (35–92)	40 (37–41)	41 (38–46)
C-reactive protein, mg/L	1.2 (0.4–4.5)	1.4 (0.5–4.7)	1.5 (0.4–4.7)
LDL cholesterol, mmol/L	1.8 (1.4–3.4)	4.0 (2.5–4.8)	2.7 (1.8–3.4)
SLCO1B1 c.521T/C[c], n	5	0	2
SLCO2B1 c.935G/A[c], n	5	2	2
UGT1A3 two *2 alleles, n	3	0	2
UGT1A3 one *2 allele, n	9	1	0
UGT1A1 c.4652T/T, n	2	0	1
UGT1A1 c.4652C/T, n	6	1	1

Statin intolerance was defined as not tolerating ≥3 statins due to muscle symptoms. Statin-dependent necrotic muscle fibers; present in biopsy after seven weeks on atorvastatin 40 mg daily, absent after eight weeks off statin.
[a] One patient was in both groups. [b] Rosuvastatin or simvastatin. [c] None of the patients were homozygous for the variant allele.
VAS; visual analogue scale (0–10 cm) for self-registered muscle symptom intensity, GFR; glomerular filtration rate, LDL; low-density lipoprotein. Results are presented as median with range, unless otherwise specified.

the sampling at T0 was excluded from the data analysis, leaving 19 patients in this group. Baseline characteristics are provided in Table 1. The group with clinical statin intolerance had higher LDL cholesterol than the tolerant group. We could not detect any differences in occurrence of the investigated genetic variants between the groups.

3.2. Creatine kinase, alanine transaminases and lactate dehydrogenase

All participants had creatine kinase (CK), alanine transaminases (ALT), and lactate dehydrogenase (LDH) within or close to the reference ranges after both the period without statins and with statins. The statin tolerant (n = 22) had statistically significant increase in absolute levels of CK, LDH, and ALT (p = 0.033, 0.031, and 0.006, respectively)

Table 2
Atorvastatin pharmacokinetic variables in patients with self-perceived SAMS and statin tolerance, statin intolerance and statin-dependent necrotic muscle fibers.

Sample material	Pharmacokinetic variable	Self-perceived SAMS > statin tolerant	Self-perceived SAMS > statin intolerant		Statin-dependent necrotic muscle fibers	
		n = 19[a]	n = 4	p-value	n = 4	p-value
Muscle T0	ATV acid	4.1 (0.7–22)	0.9 (0.6–51)	0.162	4.7 (0.6–11)	0.845
	ATV lactone	11 (2.9–21)[c]	6.6 (3.3–19)	0.434	13 (6.1–25)	0.538
	2-OH-ATV acid	5.0 (0.3–15)	0.7 (0.4–3.0)	0.027	2.4 (1.3–3.2)	0.116
	2-OH-ATV lactone	15 (5.3–32)	6.5 (2.6–14)	0.054	13 (10–17)	0.611
	4-OH-ATV acid	2.4 (0.3–20)[c]	0.7 (0.6–0.8)[e]	0.024	2.2 (0.6–4.6)	0.434
	4-OH-ATV lactone	9.2 (3.9–39)	4.8 (1.9–6.8)	0.009	7.6 (4.9–9.8)	0.324
	Sum acids	14 (3.6–53)[c]	2.2 (2.0–4.3)[e]	0.003	9.1 (4.3–18)	0.262
	Sum lactones	37 (15–63)[c]	17 (11–39)	0.042	34 (21–50)	0.652
	Ratio ATV lactone/acid	1.7 (0.9–11)[c]	5.7 (0.4–10)	0.227	2.8 (2.2–10)	0.081
	Ratio 2-OH-ATV lactone/acid	3.0 (0.5–48)	5.5 (3.4–18)	0.097	6.6 (3.4–11)	0.116
	Ratio 4-OH-ATV lactone/acid	3.5 (0.6–28)[c]	7.0 (2.5–8.0)[e]	0.471	4.9 (1.8–15)	0.837
	Ratio sum lactones/acids	2.5 (1.1–13)[d]	5.2 (5.0–5.3)[e]	0.012	3.9 (2.6–5.2)	0.172
	Acyl glucuronide	N/A	N/A	–	N/A	–
Plasma T0	ATV acid	0.7 (0.2–2.2)	0.3 (0.1–0.8)	0.116	0.8 (0.2–2.3)	0.845
	ATV lactone	1.3 (0.4–2.6)	0.7 (0.2–1.4)	0.116	1.6 (0.6–2.7)	0.505
	2-OH-ATV acid	1.2 (0.6–2.1)	0.5 (0.2–0.5)	<0.001	1.1 (0.5–2.6)	0.969
	2-OH-ATV lactone	2.3 (1.1–4.2)	1.1 (0.8–1.3)	0.003	2.6 (1.3–4.7)	0.557
	4-OH-ATV acid	0.7 (0.3–2.0)	0.3 (0.2–0.4)	0.006	0.9 (0.2–1.3)	0.907
	4-OH-ATV lactone	1.0 (0.4–3.5)	0.5 (0.5–1.6)	0.188	1.2 (0.5–1.2)	0.969
	Sum acids	2.8 (1.1–6.2)	1.1 (0.7–1.6)	0.002	2.8 (1.0–6.1)	0.969
	Sum lactones	4.8 (2.0–7.7)	2.5 (2.3–3.1)	0.016	5.3 (2.5–8.6)	0.725
	Ratio ATV lactone/acid	1.6 (1.1–3.3)	1.9 (1.5–2.6)	0.505	2.1 (1.2–2.6)	0.409
	Ratio 2-OH-ATV lactone/acid	1.9 (1.4–3.0)	2.4 (2.3–3.9)	0.044	2.2 (1.8–2.6)	0.250
	Ratio 4-OH-ATV lactone/acid	1.6 (0.4–3.4)	2.3 (1.6–4.1)	0.097	1.4 (0.9–2.7)	0.907
	Ratio sum lactones/acids	1.7 (0.9–3.2)	2.3 (1.9–3.5)	0.027	1.9 (1.4–2.6)	0.366
	Acyl glucuronide	2.0 (0.0–17)	0.5 (0.0–5.0)	0.250	2.0 (0.0–3.0)	0.456
	Ratio acyl glucuronide/ATV acid	3.8 (0.0–9.3)	1.3 (0.0–6.2)	0.286	1.4 (0.0–3.8)	0.162
Plasma T1	ATV acid	31 (9.8–103)	11 (3.5–24)	0.006	33 (19–92)	0.785
	ATV lactone	14 (3.9–82)	11 (3.9–22)	0.286	28 (9.9–50)	0.286
	2-OH-ATV acid	17 (7.1–58)	9.1 (2.4–23)	0.162	26 (8.4–32)	0.667
	2-OH-ATV lactone	12 (5.3–80)	12 (4.3–22)	0.505	26 (7.0–31)	0.286
	4-OH-ATV acid	1.5 (0.6–7.4)	0.5 (0.3–0.8)	0.002	2.0 (0.8–3.4)	0.907
	4-OH-ATV lactone	4.4 (0.9–11)	1.9 (1.4–3.5)	0.021	4.3 (2.4–6.1)	0.845
	Sum acids	49 (18–167)	21 (6.2–48)	0.035	62 (28–95)	0.725
	Sum lactones	34 (13–171)	22 (13–48)	0.286	59 (19–87)	0.366
	Ratio ATV lactone/acid	0.6 (0.2–0.9)	1.0 (0.8–1.1)	0.002	0.8 (0.5–0.9)	0.162
	Ratio 2-OH-ATV lactone/acid	0.7 (0.4–1.5)	1.4 (0.9–3.2)	0.035	0.7 (0.8–1.0)	0.324
	Ratio 4-OH-ATV lactone/acid	2.0 (1.0–9.3)	4.3 (2.9–5.3)	0.162	1.9 (1.8–4.3)	1.00
	Ratio sum lactones/acids	0.7 (0.3–1.3)	1.2 (1.0–2.1)	0.021	0.9 (0.7–1.0)	0.324
	Acyl glucuronide	80 (19–876)	48 (25–188)	0.218	235 (62–296)	0.188
	Ratio acyl glucuronide/ATV acid	2.9 (0.9–42)	6.8 (3.1–7.8)	0.116	5.7 (3.4–7.8)	0.116
Plasma T2	ATV acid	28 (7.4–76)	12 (3.2–15)	0.054	29 (12–51)	0.725
	ATV lactone	26 (7.4–78)	19 (4.1–21)	0.162	30 (20–44)	0.557
	2-OH-ATV acid	26 (7.5–47)	9.4 (2.1–14)	0.009	22 (14–32)	0.969
	2-OH-ATV lactone	37 (11–106)	20 (9.7–33)	0.116	40 (22–50)	0.907
	4-OH-ATV acid	2.5 (0.6–9.1)	0.8 (0.3–1.3)	0.016	3.6 (1.3–5.8)	0.845
	4-OH-ATV lactone	6.5 (1.4–24)	3.3 (1.3–6.0)	0.081	7.2 (3.6–8.2)	0.725
	Sum acids	51 (16–133)	24 (5.6–27)	0.027	54 (27–89)	0.785
	Sum lactones	68 (21–196)	44 (15–58)	0.116	77 (48–97)	1.00
	Ratio ATV lactone/acid	1.0 (0.6–1.8)	1.5 (1.3–1.8)	0.012	1.0 (0.9–1.8)	1.00
	Ratio 2-OH-ATV lactone/acid	1.6 (1.0–2.6)	2.4 (2.0–4.5)	0.012	1.6 (1.4–2.3)	0.785
	Ratio 4-OH-ATV lactone/acid	2.5 (0.9–5.7)	3.7 (2.8–10)	0.054	2.1 (1.2–4.6)	0.785
	Ratio sum lactones/acids	1.4 (0.8–2.5)	2.0 (0.8–2.7)	0.004	1.3 (1.1–2.1)	1.00
	Acyl glucuronide	114 (33–606)	145 (22–218)	0.845	186 (93–257)	0.785
	Ratio acyl glucuronide/ATV acid	7.5 (2.1–15.0)	13 (7.0–14)	0.067	5.8 (4.4–11)	0.907

Statin intolerance was defined as not tolerating ≥3 statins due to muscle symptoms. Necrotic muscle fibers were observed after seven weeks on atorvastatin 40 mg daily and not after eight weeks off statin. Acids and lactones in plasma are displayed as nmol/L, in muscle as pmol/g protein, and acyl glucuronide as pmol/L. ATV; atorvastatin, T0; pre-dose, T1; 1 h after dose, T2; 2 h after dose, N/A; not applicable (below limit of detection). The Mann-Whitney *U* Test was used for comparison between groups with statin-dependent muscle side effects and group with statin-independent muscle symptoms. Results are presented as median with range. a One patient lost to follow up and one patient excluded from data analysis due to ingestion of atorvastatin immediately before sample collection. b One patient in both groups. c n = 18 and d n = 17; One measurement of ATV lactone and one measurement of 4-OH-ATV acid were missing due to technical analytical reasons. e n = 3; One measurement of 4-OH-ATV acid was missing due to technical analytical reasons.

between the period without and with statins. We found poor correlations for all six atorvastatin metabolites in plasma and muscle to the CK difference measures, with Spearmans correlation coefficients of 0.085–0.331 (plasma T0), −0.098 – 0.259 (plasma T1), −0.056 – 0.477 (plasma T2), and 0.028–0.324 (muscle).

3.3. Atorvastatin pharmacokinetic variables in patients with non-SAMS, statin intolerance, and necrotic muscle fibres

Absolute levels of atorvastatin and its metabolites, as well as lactone/acid ratios in patients classified with statin tolerance, intolerance and necrotic muscle fibres, respectively, are shown in Table 2. In the T0 muscle samples, statin-intolerant patients had lower levels of three hydroxylated metabolites, and both the sum of acid compounds and the sum of lactones were lower compared to the group with statin tolerance. On the other hand, the statin-intolerant patients had a two-fold higher lactone-to-acid metabolite ratio in muscle. We found a similar pattern in blood plasma at T0, T1, and T2: lower absolute levels of several atorvastatin metabolites and 1.4–1.7-fold higher median ratios between the sum of lactones and the sum of acids. In the post-dose blood samples, the atorvastatin lactone/acid ratio and the 2-OH-atorvastatin lactone/acid ratio were 1.5 to 2-fold higher in the statin-intolerant group than in the tolerant group. The median ratio between atorvastatin acyl glucuronide and atorvastatin acid in blood plasma increased 10-fold from 1.3 at T0 to 12.6 at T2 in the statin-intolerant group ($p = 0.068$), and it increased 2-fold from 3.8 at T0 to 7.5 at T2 in the tolerant group ($p < 0.001$). In plasma, we did not observe any differences in absolute levels of atorvastatin metabolites, and the lactone/acid ratios were similar between the group with necrotic muscle fibres and the statin tolerant group. In muscle, there was a trend towards higher lactone/acid ratios in those with necrotic muscle fibres at the end of the statin treatment period.

Since both high lactone/acid ratios and low absolute levels of the acid forms were associated with clinical statin intolerance, we assessed the combination of these variables in plasma and muscle with regard to distinguishing the clinically intolerant patients in a post-hoc analysis. The cut-off values were set to distinguish the statin-intolerant patients from those who tolerated statin treatment, as illustrated in Fig. 2 and **Supplementary 1** (muscle). With 2-OH-atorvastatin lactone/acid ratio >2.15 and 2-OH-atorvastatin acid <0.65 nmol/L in plasma at T0, the sensitivity was 100 %, and the specificity was 100 %. Combining the sum of lactones/acids >1.85 and the sum of acid metabolites <1.8 nmol/L at T0 provided 100 % sensitivity and 95 % specificity. The negative predictive values of the two combinations were 100 %, and the positive predictive values were 100 % and 69 %, respectively. The diagnostic performances of the various combinations are presented in Table 3.

3.4. Specificity of derived cut-off values in an external data set

The combinatorial cut-offs were assessed against pharmacokinetic measurements from the control group without muscle complaints ($n = 39$) in the MUSE RCT study. The rate of false positives varied from 0 to 36 % (Table 3).

3.5. Impact of pharmacogenetic variants on statin metabolites

We did not observe any consistent association between the lactone/acid ratios and the gene variants in *SLCO1B1* and *SLCO2B1*. However, there was a tendency towards lower lactone/acid ratios in muscle tissue from patients with the *SLCO1B1* c.521T > C variant (heterozygotes) Supplementary 2-Table 1. Ten patients were carriers of at least one *UGT1A3**2 allele combined with at least one *UGT1A1* c.4652C > T allele. Five were *2 carriers with no T allele, whereas one was a T carrier with no*2 alleles. The levels of lactone metabolites in muscle and plasma were approximately 30 % ($p = 0.022$) and 70 % ($p = 0.003$) higher in patients with *UGT1A3* *2 and/or *UGT1A1* c.4652C > T alleles compared

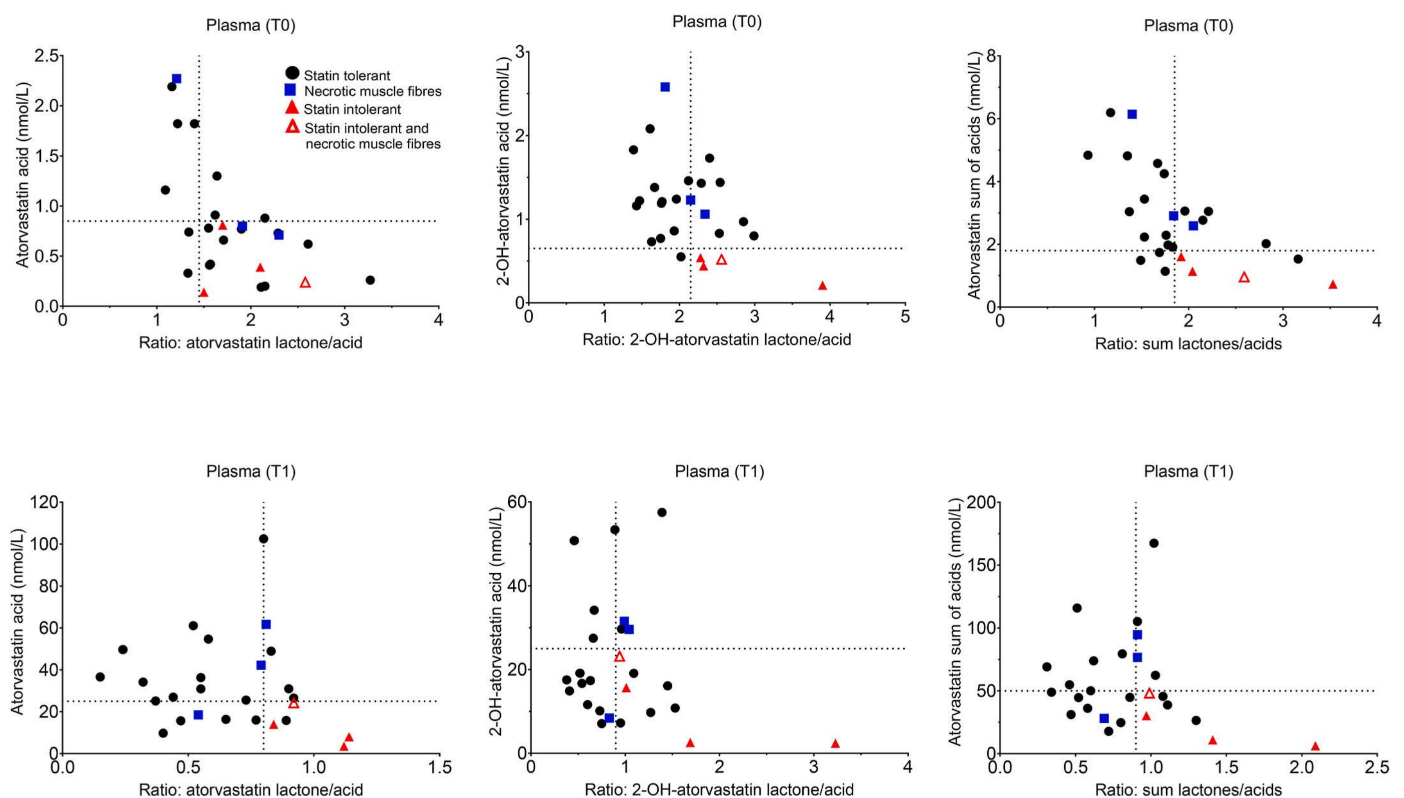

Fig. 2. Plots with combined atorvastatin acids and ratios of lactones/acids in plasma at T0 (24 h after last atorvastatin dose) and T1 (1 h after atorvastatin dose). A single symbol represents measurements of one participant. Dotted lines are cut-off values set with the purpose to differentiate the clinically statin intolerant patients (red symbols, $n = 4$) from the clinically statin tolerant (black and blue symbols, $n = 22$).

Table 3
Combined variable test performances for the differentiation of patients being clinically intolerant to statin therapy.

Sample material	Combined pharmacokinetic variables	Cut-off values	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	False positive, control group (%) (n = 39)
Plasma T0	ATV acid	<0.85	100	45	17	100	36
	ATV lactone/acid	>1.45					
	2-OH-ATV acid	<0.65	100	100	100	100	7,5
	2-OH-ATV lactone/acid	>2.15					
	Sum acid	<1.80	100	95	69	100	0
	Sum lactones/acids	>1.85					
Plasma T1	ATV acid	<25	100	95	69	100	–
	ATV lactone/acid	>0.80					
	2-OH-ATV acid	<25	100	77	33	100	–
	2-OH-ATV lactone/acid	>0.90					
	Sum acids	<50	100	86	44	100	–
	Sum lactones/acids	>0.90					

Presented cut-off values for acid form(s) and lactone/acid ratios were derived by giving priority to complete differentiation of the clinically intolerant patients (n = 4) from the patients being clinically tolerant to statin therapy (n = 22), including the patients with necrotic muscle fibres. Statin intolerance was defined as not tolerating ≥3 statins (including atorvastatin). Predictive values were calculated with 10 % prevalence of statin intolerance among patient with self-perceived statin associated muscle symptoms. The control group from MUSE RCT consisted of patients without muscle symptoms on atorvastatin treatment. The sums include acid or lactone forms of atorvastatin, 2-OH-atorvastatin and 4-OH-atorvastatin. Cut-off values for the acid metabolites are shown in nmol/L. ATV; atorvastatin, T0; pre-dose, T1; 1 h after dose.

to patients with *UGT1A1* CC and *UGT1A3* no*2 alleles, Supplementary 2-Table 2. The levels of lactone metabolites in plasma were also higher in patients carrying *UGT1A3* *2 and *UGT1A1* c.4652C > T alleles when assessed separately Supplementary 2-Table 3-4.

4. Discussion

In the patients with coronary heart disease and self-perceived SAMS, the pattern of atorvastatin metabolites differed between those with clinical statin intolerance and statin tolerance. The pattern was characterized by lower absolute levels of atorvastatin metabolites and, at the same time, a higher ratio between lactone forms and acid forms of the metabolites. This pattern was prominent both in muscle tissue and in blood plasma from patients who were clinically intolerant to statins. We did not observe a statistically significant association specifically between the pharmacokinetic variables and findings of atorvastatin-dependent necrotic muscle fibres. The combination of a high lactone/acid ratio and low acid metabolite level was promising to distinguish those with clinical statin intolerance in the present patient sample by completely separating patients with and without clinical statin intolerance. This finding was strengthened with very low rate of false positive results when we assessed the combinatorial cut-offs in the control group without any history of SAMS. The results from our study encourage further development of a biomarker for clinical statin intolerance based on metabolites of the statin, according to this diagnostic principle.

Lactone metabolites have been suggested as mediators of muscle toxicity [24]. In our study, the lactone metabolites did not appear to be associated with the side effects in terms of their absolute exposure level locally in muscle. We did not observe elevated levels of lactone metabolites in the patients who were intolerant to statins. Rather, statin intolerance due to muscle side effects was associated with decreased absolute levels of metabolites and an increased ratio between lactones and acids. The results align with a previous study, reporting a skewed lactone-to-acid balance in relation to SAMS [11]. The metabolite pattern may indicate an increased metabolic conversion rate of the acid to lactone forms. Atorvastatin acyl glucuronides are intermediates in this conversion, and increased production and turnover of acyl glucuronide metabolites might be involved in developing muscular side effects during atorvastatin therapy. Acyl glucuronide metabolites of drugs are generally considered reactive substances with the potential to induce toxic effects [25]. We were unable to support this hypothesis through our semi-quantitative determination of atorvastatin acyl glucuronide in plasma, where we also recognized that these are challenging compounds to target for pharmacokinetic observations due to the reactivity and chemical instability.

Three of four patients with necrotic muscle fibres were tolerant to statin therapy. Accordingly, a previous study reported that 10 of 14 asymptomatic, statin-treated patients had signs of damaged muscle fibres when studied morphologically using electron microscopy [26]. Importantly, the pharmacokinetic pattern in the present study was distinctly associated with clinical intolerance and not the presence of necrotic muscle fibres. Thus, the pharmacokinetic variables of statin metabolism emerge as potential diagnostic markers for SAMS in the clinical setting. Our results show that for atorvastatin, the 2-OH-atorvastatin metabolites, and the sum of lactone and acid metabolites may have the optimal potential to distinguish patients with SAMS.

Genetic variants in *UGT1A3* and *UGT1A1* were associated with higher levels of atorvastatin lactone metabolites. Nevertheless, we could not observe any trends towards an association between the assessed variants and patients with SAMS. There was considerable overlap between the presence of the examined variants in these two genes. The relevance of the separate genes and the potential relationship with SAMS should be pursued in larger data sets.

The small number of cases is a limitation of our study. However, we had a large number of controls compared to cases which is a strength of the data analyses when the cases consistently were placed at one end of the data set. Further, the atorvastatin metabolite pattern in the statin-intolerant group was present in both muscle and plasma and at different times, adding strength to our findings. Also, a higher number of controls compared to cases is a strength of the data analyses, with the cases consistently placed at one end of the data set. All participants received atorvastatin 40 mg daily, and the results cannot be directly extrapolated to other dose levels. Therefore, the presented results need validation in a larger study with patients intolerant to statins.

Our study proposes developing a simple blood sample test to accurately distinguish patients with atorvastatin-dependent muscle symptoms from those with muscle symptoms caused by other factors. Such a laboratory test would substantially support clinical decisions and decrease the time and resources spent on statin de- and -re-challenge procedures. The measurements of atorvastatin and its metabolites have yet to be widely established; however, the LC-MS/MS instruments are standard equipment in many hospital laboratories. The analyses based on a single-timed blood sample could be available for clinicians like other standard pharmacological analyses. This prospect requires confirmation and validation in a larger study, including different dosages.

5. Conclusion

Our exploratory, case-control study indicates a distinct pattern of atorvastatin metabolites in patients with coronary heart disease being clinically intolerant to statin therapy due to muscle symptoms. In statin-intolerant patients, we observed lower absolute levels of atorvastatin metabolites and a higher ratio between lactone and acid forms of the metabolites, both in muscle tissue and blood plasma. The combination of a high lactone/acid ratio and a low absolute level of corresponding acid metabolite demonstrated the potential for a biomarker test with high diagnostic accuracy to distinguish between patients with and without SAMS. If the concept can be further confirmed and developed into clinical applicability, it may become a valuable tool in the follow-up of patients who report SAMS.

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Author contribution

Trine Lauritzen: Writing – original draft, Investigation, Formal analysis, Visualization. John Munkhaugen: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration. Stein Bergan: Conceptualization, Writing – review & editing, Supervision. Kari Peersen: Investigation, Writing – review & editing. Anja Camilla Svarstad: Investigation, Writing – review & editing. Anders M Andersen: Investigation, Writing – review & editing. Jens Pahnke: Investigation, Writing – review & editing. Einar Husebye: Conceptualization, Methodology, Writing – review & editing. Nils Tore Vethe: Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JM reports having received modest lecture fees from Novartis and Bayer, outside the submitted work. The other authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2024.01.001.

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