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

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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
1 | INTRODUCTION

Poor adherence to statin treatment is a prevalent challenge in cardiovascular disease prevention, associated with adverse outcomes. Regular assessment of adherence is recommended by the European lipid guideline from 2016, and was recently given a class IA recommendation in the corresponding US guideline. 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. Prescription fill rates obtained from pharmacy registries provides the most comprehensive data on statin adherence today. 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. Hence, objective methods to detect reduced adherence are requested 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. Assays for measuring statins and metabolites in blood with direct chromatography and tandem mass spectrometry (LC–MS/MS) methods have been described. However, they are generally designed for studies on pharmacokinetics and bioequivalence. We have recently reported a fast and reliable assay for the quantification of atorvastatin and its 5 major metabolites with LC–MS/MS methodology. Importantly, this assay is feasible for the routine clinical laboratory with respect to technical implementation and interpretation of adherence.

Atorvastatin is the most frequently used statin for the prevention of coronary heart disease (CHD) in Europe and Norway. 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 and the enzyme activity shows high variability between individuals. 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.

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, that adherence could be discriminated from partial adherence after the dose had been omitted for 1 to 3 days.

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 (t0) 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 (t24), 48 (t48), 72 (t72) and 96 (t96) 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, t1 and t2).

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 classified 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 t24, t48, and t72. 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 accordance with ICH/Good Clinical Practice. The study protocol was reviewed 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 t0 and mean 96% (range 69–150%, 95% CI 81–111%) at t94.

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.

3.2 | Factors associated with the drug exposure

Increasing age was associated with increasing dose-normalized trough concentrations of atorvastatin acid (t0); 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 side effects were not associated with significant variations in atorvastatin and metabolites concentrations at t0.

Compared with patients expressing the wild types, the mean dose-normalized t0 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

| 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 |

| 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 | 0.725 | 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 t0 samples handled according to the low temperature procedure. ATV, atorvastatin; 2OH, ortho-hydroxy; 4OH, para-hydroxy.
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 (3.3–14) days at 40 mg, and 5.6 (3.8–20) days at 80 mg. These pharmacokinetic simulations were based on linear regression with R² 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).

3.5 | Identification of an unscheduled dose prior to blood sampling

The maximum dose-normalized 2-component sum at t₀ 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, t₁ and 2.21 (range 0.09–5.64) nM/mg at day 4, t₂. At day 4, t₁ and t₂, 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. 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 inflammation28 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. 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.

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