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Risk of misclassification with a non-fasting lipid profile in secondary cardiovascular prevention

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ABSTRACT

Aims: Routinely fasting is not necessary for measuring the lipid profile according to the latest European consensus. However, LDL-C tends to be lower in the non-fasting state with risk of misclassification. The extent of misclassification in secondary cardiovascular prevention with a non-fasting lipid profile was investigated.

Methods and results: 329 patients on lipid lowering therapy for secondary cardiovascular prevention measured a fasting and non-fasting lipid profile. Cut-off values for LDL-C, non-HDL-C and apolipoprotein B were set at < 1.8 mmol/l, < 2.6 mmol/l and < 0.8 g/l, respectively. Study outcomes were net misclassification with non-fasting LDL-C (calculated using the Friedewald formula), direct LDL-C, non-HDL-C and apolipoprotein B. Net misclassification < 10% was considered clinically irrelevant. Mean age was 68.3 ± 8.5 years and the majority were men (79%). Non-fasting measurements resulted in lower LDL-C (− 0.2 ± 0.4 mmol/l, $P < 0.001$), direct LDL-C (− 0.1 ± 0.2 mmol/l, $P = 0.001$), non-HDL-C (− 0.1 ± 0.4 mmol/l, $P = 0.004$) and apolipoprotein B (− 0.02 ± 0.10 g/l, $P = 0.004$). 36.0% of the patients reached a fasting LDL-C target of < 1.8 mmol/l with a significant net misclassification of 10.7% (95% CI 6.4–15.0%) in the non-fasting state. In the non-fasting state net misclassification with direct LDL-C was 5.7% (95% CI 2.1–9.2%), 4.0% (95% CI 1.0–7.4%) with non-HDL-C and 4.1% (95% CI 1.1–9.1%) with apolipoprotein B.

Conclusion: Use of non-fasting LDL-C as treatment target in secondary cardiovascular prevention resulted in significant misclassification with subsequent risk of undertreatment, whereas non-fasting direct LDL-C, non-HDL-C and apolipoprotein B are reliable parameters.

1. Introduction

Current European and US guidelines for the management of dyslipidemia recommend to measure the lipid profile in the fasting state [1,2]. However, a recent consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine suggests that fasting is not routinely required for determination of a lipid profile [3]. In addition, the British National Clinical guideline states that a fasting sample is not needed and the new Canadian guideline acknowledges a non-fasting lipid profile as a suitable alternative to a fasting lipid profile [4,5]. The non-fasting determination of blood lipids has several advantages. First, patients are allowed to eat normally before blood sampling and it allows physicians to determine the lipid profile at any random moment. In addition, it may prevent long waiting times for blood sampling in the early morning and the risk of hypoglycemic events in patients with diabetes mellitus [6]. Large-scale population studies and registries have shown that the lipid profile is only minimally affected after habitual food intake. Total cholesterol, LDL-C and non-HDL-C are approximately decreased with 0.2 mmol/l, whereas triglycerides are increased with 0.3 mmol/l. Both apolipoprotein B and HDL-C remain largely unaffected [3,7–11]. However, these studies were unpaired and nonrandomized. In contrast, diurnal measurements of triglycerides have shown that triglycerides increase approximately 0.5 mmol/l in women and 1.0 mmol/l in men, which may significantly affect LDL-C when calculated using the Friedewald equation [12].

The decrease in LDL-C and non-HDL-C after habitual food intake may result in potential misclassification. It has been suggested that the

Abbreviations: T2DM, type 2 diabetes mellitus; PCSK9, proprotein convertase subtilisin-kexin 9; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

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risk of misclassification due to habitual food intake will be minimal and without clinical consequences, but precautions are given for patients with diabetes mellitus [3]. After the intake of a standardized fat-rich breakfast, 38% of the patients with diabetes mellitus were reclassified in a lower risk category when LDL-C was used [13]. The current European guideline advices to intensify lipid lowering therapy in patients with cardiovascular disease (very high risk) to reach LDL-C levels < 1.8 mmol/l. Non-HDL-C and apolipoprotein B may be used as secondary treatment targets set at 2.6 mmol/l and 0.8 g/l, respectively, in patients with cardiovascular disease [1]. No data exist concerning misclassification due to habitual food intake in patients with cardiovascular disease on lipid lowering therapy and subsequent risk of undertreatment [14]. We studied the risk of misclassification with non-fasting LDL-C, direct LDL-C, non-HDL-C and apolipoprotein B in patients with cardiovascular disease on stable lipid lowering therapy in a randomized, cross-over study. An upper margin of 10% net misclassification was regarded as clinically irrelevant.

2. Materials and methods

2.1. Subjects

Participants were recruited from the outpatient clinics of Internal Medicine and Cardiology, Albert Schweitzer Hospital in Dordrecht, the Netherlands. The inclusion was carried out between October 2015 and November 2016. All subjects were known with cardiovascular disease and were on stable lipid lowering therapy for secondary cardiovascular prevention. Cardiovascular disease was defined as the presence of at least one of the following conditions before inclusion: a myocardial infarction, angina pectoris based on clinical characteristics, documented coronary artery disease based on a coronary angiogram, a cerebral infarction, transient ischemic attack or the presence of peripheral artery disease. Lipid lowering therapy was defined as the use of a statin, ezetimib, fibrate, nicotinic acid, proprotein convertase subtilisin-kexin 9 (PCSK9) inhibitor or a combination of these. Exclusion criteria were an age below 18 years and changes in lipid lowering therapy within the last four weeks. Anthropometric characteristics, e.g. weight, length and body mass index (BMI) were recorded.

2.2. Study design

The study was designed as an open, randomized, cross-over study. Subjects were randomized between determining a fasting lipid profile first followed by a second lipid profile after habitual food intake on another day (group A) or vice versa (group B). Fasting subjects were not allowed to eat from 11:00 pm the prior evening until the fasting blood test, but subjects were allowed to drink water or tea without additions like sugar or milk. Subjects were instructed to have their daily routine concerning habitual food intake when obtaining the non-fasting blood test. Subjects were free to choose when the blood samples were taken during office hours: from 7:30 am until 5:00 pm, but both blood tests had to be obtained within 21 days from each other. Subjects were not allowed to measure both the fasting and non-fasting sample on the same day. All subjects provided written informed consent. The study was approved by the independent Regional Ethical Committee Rotterdam, The Netherlands, and was registered at the Dutch Trial Register (NTR5321).

2.3. Laboratory measurements

All clinical and hematological chemistry measurements were carried out on freshly drawn blood samples and analyzed at Result Laboratorium, Dordrecht, the Netherlands, except for apolipoprotein B. Albumin, glucose, total cholesterol, HDL-C and triglycerides were determined using a Dimension VISTA 1500 System (Siemens Healthcare, Germany). LDL-C was calculated using the Friedewald equation when triglycerides were < 4.00 mmol/l. Non-HDL-C was calculated by subtracting HDL-C from total cholesterol. Direct LDL-C was measured with a homogeneous assay without the need for any off-line pretreatment of centrifugation steps (Dimension Vista system, Siemens, München, Germany). The method was in a two reagent format and depended on the properties of a specific detergent that solubilizes only non-LDL-particles. The cross-reactivity with HDL-C was estimated to be maximal 1.2%. Day-to-day CV's on the Dimension Vista were 2.0% for LDL-C at concentrations of both 2.82 and 3.75 mmol/l. Apolipoprotein B was determined by nephelometry using a Behring Nephelometer Analyzer II (Behring, King of Prussia, Pennsylvania, United States of America) with a commercially available kit (Siemens). Blood cell counts including hemoglobin and hematocrit were determined automatically using Sysmex XN-9000 hematology analyzers (Sysmex Corporation, Kobe, Japan).

2.4. Selection of lipid cut-off values

Cut-off values for LDL-C, direct LDL-C, non-HDL-C and apolipoprotein B were selected using the European guideline for the management of dyslipidemia [1], which advises a treatment target of < 1.8 mmol/l for both LDL-C and direct LDL-C in patients with known cardiovascular disease. Therefore, we have chosen a cut-off value of < 1.8 mmol/l as our primary cut-off value for both LDL-C and direct LDL-C. The treatment target for non-HDL-C is 0.8 mmol/l on top of the LDL-C target, which resulted in a non-HDL-C primary cut-off value of < 2.6 mmol/l. The treatment target and chosen cut-off value for apolipoprotein B was 0.8 g/l.

2.5. Statistics

Both study groups were analyzed separately and compared with each other in order to investigate relevant differences between the two study groups. In addition, data from both study groups were combined together to reach the proposed statistical power regarding risk of misclassification. The mean ± standard deviation is shown in the text and tables unless stated otherwise. Differences in continuous variables between fasting and non-fasting samples were tested using the paired Student's *t*-test. Triglycerides and the delay between fasting and non-fasting blood tests were logarithmically transformed before analysis, but non-transformed data are shown in the text and tables. Categorical data were tested with the Chi-square test. Net misclassification with non-fasting samples was determined by subtracting the number of patients below treatment target in the fasting state from the number of patients below treatment target in the non-fasting state. The 95% confidence interval (CI) for net misclassification was calculated using the bootstrap method. We concluded that a net misclassification of 10% for non-fasting LDL-C, direct LDL-C, non-HDL-C and/or apolipoprotein B would still be acceptable for clinical practice. Therefore, a net misclassification of < 10% was defined as non-inferior. We have chosen the net misclassification as primary endpoint since it corrects mostly for the intra-individual variability and intra-assay variation. Using previous data [15] a sample size of 316 subjects was needed to obtain 90% power for a one sided exact binomial test of size 2.5% against the null hypothesis of the percentage of misclassified patients being larger than 10%. Sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV) were calculated for non-fasting samples concerning the cut-off values. All statistical analyses were performed using SPSS version 22.0 (IBM SPSS Statistics, New York, United States).

3. Results

3.1. Patient characteristics

A total of 369 patients provided written informed consent, but 40

Table 1
Patient characteristics for study groups A and B and total study population. There were no significant differences in patient characteristics between study groups A and B.

	Study group A (N = 157)	Study group B (N = 172)	Total study population (N = 329)
Age (years)	69.2 ± 7.9	67.5 ± 8.9	68.3 ± 8.5
Male gender (%, N)	78.3% (123)	79.7% (137)	79% (260)
Body mass index (kg/m^2)	27.5 ± 4.2	28.1 ± 4.4	27.8 ± 4.3
Coronary artery disease (%, N)	82.8% (130)	82.6% (142)	82.7% (272)
Cerebrovascular disease (%, N)	22.3% (35)	23.3% (40)	22.8% (75)
Peripheral artery disease (%, N)	11.5% (18)	12.8% (22)	12.2% (40)
Diabetes mellitus (%, N)	22.9% (36)	25.0% (43)	23.9% (78)
Use of insulin (%, N)	10.2% (16)	15.1% (26)	12.8% (42)
Use of statin (%, N)	96.8% (152)	97.7% (168)	97.3% (320)
Use of ezetimib (%, N)	7.0% (11)	11.0% (19)	9.1% (30)
Use of fibrate (%, N)	1.3% (2)	0.6% (1)	0.9% (3)
Use of nicotinic acid (%, N)	0.0% (0)	0.0% (0)	0.0% (0)
Use of PCSK9-inhibitor (%, N)	0.0% (0)	0.0% (0)	0.0% (0)

patients were excluded from the analyses: one patient did not use a lipid lowering drug, another patient started a new lipid lowering drug between the two blood tests and 38 other patients did not complete both the fasting and non-fasting blood test. The remaining 329 patients were included in the analyses of whom 157 patients (47.7%) were randomized to measure the first blood test in the fasting state (group A), whereas 172 patients (52.3%) were randomized to measure the first blood test in the non-fasting state (group B). Patient characteristics of groups A and B and the total study population are shown in Table 1. There were no significant differences in patient characteristics between groups A and B. Mean age was 68.3 ± 8.5 years and the majority were men (79%). All patients were known with cardiovascular disease and 23.9% were known with diabetes mellitus. A total of 320 patients used a statin as lipid lowering therapy (97.3%) and 30 patients (9.1%) used ezetimib as either stand-alone lipid-lowering therapy or in combination with a statin. Only three patients (0.9%) used a fibrate and none a PCSK-9 inhibitor or nicotinic acid (Table 1).

3.2. Distribution of blood tests

The median number of days between the fasting and non-fasting samples was comparable between groups A and B: 7 days (IQR 3–13) for group A and 7 days (IQR 3–12) for group B (P = 0.61). Patients choose to have their non-fasting blood samples drawn at a significantly later time with similar results in both groups (median 8:00 am (IQR 8:00 am–9:00 am) for fasting samples versus median 11:00 am (IQR 10:00 am–01:00 pm) for non-fasting samples, P < 0.001 for both groups A and B) with a median difference between fasting and non-fasting samples of 153 min (IQR 54–270) for group A and 149 min (IQR 76–308) for group B (P = 0.41). Fig. 1 shows the distribution of the fasting and non-fasting samples over the day for the total study population.

3.3. Differences in lipid parameters between the fasting and non-fasting state

Lipid parameters were comparable between groups A and B in both the fasting and non-fasting state, except for HDL-C, which was slightly lower in group B. The mean difference between fasting and non-fasting samples for total cholesterol, LDL-C and HDL-C was slightly higher in group B compared to group A. Although statistically significant the absolute differences were only minor and all other parameters were

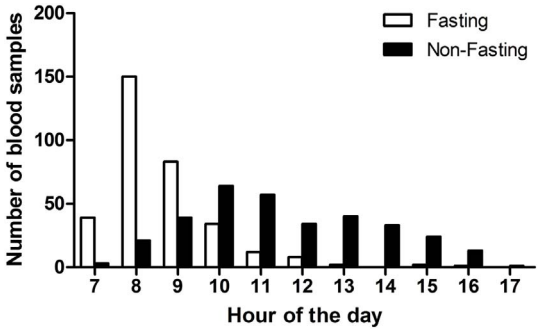

Fig. 1. Distribution of the fasting and non-fasting blood samples per hour of the day in the total study population (N = 329).

comparable between both groups (Table 2). Overall, non-fasting measurements resulted in lower LDL-C (− 0.2 ± 0.4 mmol/l), direct LDL-C (− 0.1 ± 0.2 mmol/l), non-HDL-C (− 0.1 ± 0.4 mmol/l) and apolipoprotein B (− 0.02 ± 0.10 g/l). Triglycerides were 0.35 ± 0.62 mmol/l higher in the non-fasting state (Table 3). LDL-C could not be calculated in the fasting state due to hypertriglyceridemia > 4.00 mmol/l in 10 patients (3.0%), which was comparable to the number of patients in the non-fasting state (19 patients, 5.8%; P = 0.18).

3.4. Misclassification in the non-fasting state

No significant differences in misclassification with LDL-C, direct LDL-C, non-HDL-C and apolipoprotein B were found between groups A and B (Table 4). A total of 36.0% of the patients in the total study population reached an LDL-C target of < 1.8 mmol/l in the fasting state in comparison to 46.8% in the non-fasting state, which resulted in a net misclassification of 10.7% (95% CI 6.4–15.0%). The number of patients reaching an LDL-C target of < 1.8 mmol/l was lower when using direct LDL-C: 27.0% in the fasting state and 32.6% in the non-fasting state. Net misclassification in the non-fasting state was lower with direct LDL-C in comparison to LDL-C (5.7% versus 10.7%; P < 0.001). Net misclassification in the non-fasting state with non-HDL-C and apolipoprotein B was significantly lower compared to net misclassification with LDL-C (P < 0.001 and P = 0.002, respectively). Net misclassification for non-HDL-C with a cut-off value < 2.6 mmol/l was 4.0% (95% CI 1.0–7.4%) and 4.1% (95% CI 1.1–9.1%) for apolipoprotein B with a cut-off value < 0.80 g/l (Table 4). Net misclassification with non-fasting LDL-C, direct LDL-C, non-HDL-C and apolipoprotein B was comparable for patients with and without diabetes mellitus.

3.5. Sensitivity, specificity, positive predictive value and negative predictive value

Overall, the sensitivity for non-fasting LDL-C, direct LDL-C, non-HDL-C and apolipoprotein B was above 0.90 for every lipid parameter and chosen cut-off values. The specificity differed between lipid parameters and according to the cut-off value used. Specificity was best for non-fasting direct LDL-C < 1.8 mmol/l and non-HDL-C < 2.5 mmol/l. Non-fasting LDL-C < 1.8 mmol/l and direct LDL-C < 1.8 mmol/l showed the lowest PPV, but also the highest NPV. Non-fasting non-HDL-C < 2.5 mmol/l and apolipoprotein B < 0.8 g/l showed the best overall performance regarding the PPV and NPV (Table 5).

4. Discussion

To our knowledge this is the first study to address the exact risk of misclassification with a non-fasting lipid profile in patients with cardiovascular disease who receive lipid lowering drugs for secondary cardiovascular prevention. The use of non-fasting LDL-C resulted in net misclassification of 10.7% of the subjects with potential risk of undertreatment despite only small absolute changes in LDL-C concentration.

Table 2
Laboratory measurements in the fasting and non-fasting state are shown for group A (N = 157) and B (N = 172) including their corresponding mean difference. Data are given as mean ± SD except for triglycerides, which are shown as median with IQR.

		Group A (N = 157)	Group B (N = 172)	P-value
Total cholesterol (mmol/l)	Fasting	4.1 ± 0.7	4.1 ± 0.9	0.89
	Non-fasting	4.1 ± 0.8	4.0 ± 0.8	0.26
	Mean difference	−0.0 ± 0.4	−0.1 ± 0.4	0.01
LDL-C (mmol/l)	Fasting	2.1 ± 0.7	2.1 ± 0.8	0.91
	Non-fasting	2.0 ± 0.7	1.9 ± 0.7	0.28
	Mean difference	−0.1 ± 0.4	−0.2 ± 0.4	0.03
Direct LDL-C (mmol/l)	Fasting	2.3 ± 0.6	2.3 ± 0.7	0.62
	Non-fasting	2.2 ± 0.6	2.2 ± 0.7	0.95
	Mean difference	−0.0 ± 0.2	−0.1 ± 0.3	0.12
HDL-C (mmol/l)	Fasting	1.25 ± 0.33	1.17 ± 0.28	0.02
	Non-fasting	1.25 ± 0.35	1.13 ± 0.27	< 0.001
	Mean difference	0.00 ± 0.13	−0.04 ± 0.11	0.001
Non-HDL-C (mmol/l)	Fasting	2.8 ± 0.7	2.9 ± 0.9	0.31
	Non-fasting	2.8 ± 0.8	2.8 ± 0.8	0.79
	Mean difference	−0.0 ± 0.3	−0.1 ± 0.4	0.09
Apolipoprotein B (g/l)	Fasting	0.77 ± 0.18	0.80 ± 0.22	0.17
	Non-fasting	0.77 ± 0.19	0.78 ± 0.21	0.51
	Mean difference	−0.01 ± 0.09	−0.03 ± 0.10	0.07
Triglycerides (mmol/l)	Fasting	1.40 (1.10–2.10)	1.50 (1.10–2.20)	0.23
	Non-fasting	1.80 (1.30–2.40)	1.90 (1.50–2.60)	0.10
	Mean difference	0.32 ± 0.59	0.37 ± 0.65	0.47
Glucose (mmol/l)	Fasting	6.4 ± 1.5	6.7 ± 1.7	0.12
	Non-fasting	6.8 ± 1.9	7.1 ± 2.6	0.17
	Mean difference	0.4 ± 1.7	0.4 ± 2.2	0.81
Hemoglobin (mmol/l)	Fasting	8.7 ± 1.0	8.8 ± 0.9	0.10
	Non-fasting	8.6 ± 1.0	8.8 ± 0.8	0.05
	Mean difference	−0.1 ± 0.3	−0.0 ± 0.3	0.44
Hematocrit (l/l)	Fasting	0.427 ± 0.045	0.434 ± 0.038	0.13
	Non-fasting	0.424 ± 0.045	0.430 ± 0.037	0.15
	Mean difference	−0.004 ± 0.015	−0.004 ± 0.015	0.84
Albumin (g/l)	Fasting	37.1 ± 2.5	37.5 ± 2.3	0.11
	Non-fasting	37.2 ± 2.6	37.6 ± 2.4	0.19
	Mean difference	0.1 ± 1.6	−0.0 ± 1.8	0.71

Table 3
Laboratory measurements in the fasting and non-fasting state for the total study group (N = 329). Data are shown as mean ± SD, except for triglycerides, which are shown as median [IQR].

	Fasting	Non-fasting	Mean difference	P-value
Total cholesterol (mmol/l)	4.1 ± 0.8	4.0 ± 0.8	−0.1 ± 0.4	< 0.001
LDL-C (mmol/l)	2.1 ± 0.7	1.9 ± 0.7	−0.2 ± 0.4	< 0.001
Direct LDL-C (mmol/l)	2.3 ± 0.6	2.2 ± 0.6	−0.1 ± 0.2	0.001
HDL-C (mmol/l)	1.21 ± 0.31	1.19 ± 0.32	−0.02 ± 0.12	0.001
Non-HDL-C (mmol/l)	2.9 ± 0.8	2.8 ± 0.8	−0.1 ± 0.4	0.004
Apolipoprotein B (g/l)	0.79 ± 0.20	0.77 ± 0.20	−0.02 ± 0.10	0.004
Triglycerides (mmol/l)	1.50 [1.10–2.20]	1.80 [1.40–2.50]	0.35 ± 0.62	< 0.001
Glucose (mmol/l)	6.6 ± 1.6	7.0 ± 2.3	0.4 ± 1.9	< 0.001
Hemoglobin (mmol/l)	8.8 ± 0.9	8.7 ± 0.9	−0.1 ± 0.3	< 0.001
Hematocrit (l/l)	0.431 ± 0.041	0.427 ± 0.041	−0.004 ± 0.015	< 0.001
Albumin (g/l)	37.4 ± 2.4	37.4 ± 2.5	0.0 ± 1.7	0.87

The risk of misclassification of 10.7% with non-fasting LDL-C was comparable to two other studies, which investigated the risk of misclassification of non-fasting LDL-C, but specifically in patients with diabetes mellitus [13,16]. Therefore, misclassification with non-fasting LDL-C is not only a risk in patients with diabetes mellitus, but in all patients with cardiovascular disease.

Absolute changes between fasting and non-fasting LDL-C were only 0.2 mmol/l. This was reflected by a relatively low specificity for non-fasting LDL-C. Sensitivity for non-fasting LDL-C was high, which suggests that overtreatment due to non-fasting measurements will rarely occur. Only 36.0% of our study population was appropriately treated according to the European LDL-C treatment target of < 1.8 mmol/l. Other studies showed comparable results with only a minority of patients who were adequately treated to lipid targets [17,18]. Therefore, it may be argued that much more benefit can be achieved by adequately intensifying lipid lowering therapy in the remaining subjects rather than to focus on whether or not fasting is necessary for obtaining a reliable lipid profile.

The risk of misclassification was significantly lower with non-fasting direct LDL-C, non-HDL-C and apolipoprotein B when compared to non-fasting LDL-C. Net misclassification with non-fasting direct LDL-C, non-HDL-C and apolipoprotein B did not exceed 10% and is therefore expected not to be clinically relevant. The small net misclassification with direct LDL-C, non-HDL-C and apolipoprotein B could probably be explained by slight hemodilution in the non-fasting state since hemoglobin and hematocrit were slightly decreased in the non-fasting state, although albumin remained unchanged [9]. Our study showed that direct LDL-C, non-HDL-C and apolipoprotein B are more reliable parameters than LDL-C when measured non-fasting. In addition, non-HDL-C and apolipoprotein B reflect the total burden of atherogenic

Table 4
Number of patients reclassified and net misclassified per pre-specified treatment target per lipid parameter in the non-fasting state. Data are shown for group A (N = 157), group B (N = 172) and the total study group (N = 329).

		Fasting target reached	Non-fasting target reached	Reclassification in the non-fasting state		Net misclassification in the non-fasting state	
				Below target	Above target	Mean	95% CI
LDL-C < 1.8 mmol/l	Group A	32.9% (49)	41.3% (62)	11.4% (17)	2.7% (4)	8.7%	–
	Group B	39.0% (62)	51.6% (82)	15.1% (24)	2.5% (4)	12.6%	–
	Total group	36.0% (111)	46.8% (144)	13.3% (41)	2.6% (8)	10.7%	6.4–15.0%
Direct LDL-C < 1.8 mmol/l	Group A	26.1% (41)	31.4% (49)	6.5% (10)	1.3% (2)	5.2%	–
	Group B	28.3% (47)	33.9% (58)	9.7% (16)	3.6% (6)	6.1%	–
	Total group	27.0% (86)	32.6% (104)	8.2% (26)	2.5% (8)	5.7%	2.1–9.2%
Non-HDL-C < 2.6 mmol/l	Group A	40.1% (63)	45.2% (71)	7.6% (12)	2.5% (4)	5.1%	–
	Group B	43.0% (74)	46.2% (79)	6.4% (11)	3.5% (6)	2.9%	–
	Total group	41.8% (137)	45.7% (150)	7.0% (23)	3.0% (10)	4.0%	1.0–7.4%
Apolipoprotein B < 0.8 g/l	Group A	55.6% (85)	58.7% (91)	8.6% (13)	5.3% (8)	3.3%	–
	Group B	52.4% (87)	57.4% (97)	9.8% (16)	3.1% (5)	6.7%	–
	Total group	53.8% (169)	58.9% (185)	9.2% (29)	4.1% (13)	4.1%	1.1–9.1%

Table 5
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of lipid treatment targets when using non-fasting samples compared to fasting samples for the total study population (N = 329).

	Sensitivity	Specificity	PPV	NPV
LDL-C < 1.8 mmol/l	0.93	0.79	0.72	0.95
Direct LDL-C < 1.8 mmol/l	0.90	0.89	0.75	0.96
Non-HDL-C < 2.6 mmol/l	0.93	0.88	0.85	0.94
Apolipoprotein B < 0.8 g/l	0.92	0.80	0.84	0.90

cholesterol and circulating atherogenic lipoproteins, respectively, including chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) besides low density lipoproteins (LDL) and reflect the total burden of atherogenic lipoproteins better than LDL-C [6].

Direct LDL-C was higher when compared to LDL-C, which was calculated using the Friedewald equation. Therefore, a smaller proportion of the study population reached the treatment targets when using direct LDL-C in comparison to LDL-C. Others have made similar observations, but controversy remains whether direct LDL-C overestimates cardiovascular risk or whether the Friedewald equation underestimates cardiovascular risk [19,20]. Misclassification with non-fasting direct LDL-C was low, although the PPV of direct LDL-C with a cut-off value < 1.8 mmol/l was as low as for LDL-C. The increase in direct LDL-C concentrations in combination with a concomitant low number of subjects who reached the treatment target may have contributed to the low PPV for direct LDL-C.

Patients were free to choose when the blood samples were taken during the day. This reflects daily routine clinical practice most accurately. Patients preferred to have their blood drawn in the early morning when it was necessary to fast with half of all fasting blood draws between 8:00 am and 9:00 am. This natural preference of patients may cause long waiting queues at the laboratory. This may be prevented when lipid profiles are determined non-fasting instead of fasting. Our study clearly showed that blood sampling was much more evenly distributed over the day when there was no need to fast, which is advantageous for patients and hospital logistics.

A limitation of the study is the lack of multiple sampling for both fasting and non-fasting lipid profiles. Therefore, it is impossible to calculate the intra-individual variability per lipid parameter. It would have been informative which part of the reclassification was exactly caused by the intra-individual variability and intra-assay variation. The use of net misclassification has probably corrected for most of the intra-individual variability and assay variations, which can as high as 12% for LDL-C using the Friedewald equation [21,22]. However, the results of sensitivity, specificity, PPV and NPV must have been influenced in part by the intra-individual variability and intra-assay variation. Another limitation of the study is the lack of data whether patients would have been treated differently with lipid lowering drugs when treatment would have actually been based on non-fasting lipid profiles instead of fasting lipid profiles. A randomized controlled trial with follow-up will be necessary to provide this answer. None of the subjects used a PCSK9-inhibitor, probably since this drug class has only recently been introduced into clinical practice. PCSK9-inhibitors have shown to lower LDL-C efficiently on top of statins [23,24]. It is expected that the introduction of PCSK9-inhibitors will result in a higher number of patients correctly treated to treatment targets, which may affect the extent of misclassification with non-fasting LDL-C and the other lipid parameters. It is questionable whether the clinical implications of our results will remain applicable when PCSK9-inhibitors will have widespread use in patients with cardiovascular disease. Finally, our results cannot be extrapolated to the general population and screening for primary cardiovascular prevention since our study was performed specifically in patients, which already used lipid lowering drugs as secondary cardiovascular prevention. Therefore, it is also unknown whether the use non-fasting lipid profiles will have impact on adequate diagnosing primary lipid disorders. Future studies will be necessary to address these topics.

5. Conclusions

The use of non-fasting LDL-C as treatment target in secondary cardiovascular prevention results in significant misclassification with subsequent risk of undertreatment. Non-fasting direct LDL-C, non-HDL-C and apolipoprotein B are more reliable parameters as treatment target in secondary cardiovascular prevention with negligible risk of misclassification. However, more benefit can be achieved by adequately intensifying lipid lowering therapy in patients with cardiovascular disease with suboptimal lipid levels rather than to focus on whether or not fasting is necessary for reliable lipid measurements.

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Conflict of interest

None declared.

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