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Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group

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ABSTRACT

Background: Despite international standardization programs for LDLc and HDLc measurements, results vary significantly with methods from different manufacturers. We aimed to simulate the impact of analytical error and hypertriglyceridemia on HDLc- and LDLc-based cardiovascular risk classification.
Methods: From the Dutch National EQA-2012 external quality assessment of 200 clinical laboratories, we examined data from normotriglyceridemic (~1 mmol/l) and hypertriglyceridemic (~7 mmol/l) serum pools with lipid target values assigned by the Lipid Reference Laboratory in Rotterdam. HDLc and LDLc were measured using direct methods of Abbott, Beckman, Siemens, Roche, Olympus, or Ortho Clinical Diagnostics. We simulated risk reclassification using HDL- and sex-specific SCORE multipliers considering two fictitious moderate-risk patients with initial SCORE 4% (man) and 3% (woman). Classification into high-risk treatment groups (LDLc >2.50 mmol/l) was compared between calculated LDLc and direct LDLc methods.
Results: Overall HDLc measurements in hypertriglyceridemic serum showed negative mean bias of −15%. HDL-multipliers falsely reclassified 70% of women and 43% of men to a high-risk (SCORE >5%) in hypertriglyceridemic serum ($P < 0.0001$ vs. normotriglyceridemic serum) with method-dependent risk reclassifications. Direct LDLc in hypertriglyceridemic serum showed positive mean bias with Abbott (+16%) and Beckman (+14%) and negative mean bias with Roche (−7%). In hypertriglyceridemic serum, 57% of direct LDLc measurements were above high-risk treatment goal (2.50 mmol/l) vs. 29% of direct LDLc (33% of calculated LDLc) in normotriglyceridemic sera.
Conclusion: LDLc and HDLc measurements are unreliable in severe hypertriglyceridemia, and should be applied with caution in SCORE risk classification and therapeutic strategies.

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1. Introduction

The EAS/ESC guidelines recommend that asymptomatic individuals at high cardiovascular disease (CVD) mortality risk should be identified for statin therapy [1]. For this purpose, risk assessment is performed using the SCORE (Systematic COronary Risk Evaluation) prediction model estimating 10-y risk of CVD mortality, based on gender, age, total cholesterol, systolic blood pressure and smoking status. Recently, the 2011 ESC–EAS guidelines on the management of dyslipidemias have considered the additional impact of high-density lipoprotein cholesterol (HDLc) on CVD risk by displaying 4 separate SCORE charts to 4 different levels of HDLc (mmol/l): 0.8, 1.0, 1.4 and 1.8 [1]. The effects of differing HDLc levels may also be calculated from the classical SCORE using HDL- and sex-specific multipliers according to Descamps et al. [2].

In patients with dyslipidemia, prevention strategies with either lifestyle changes or lipid-lowering agents are primarily targeted by low-density lipoprotein cholesterol (LDLc). The higher the predicted risk, the lower is the recommended LDLc goal and hence the need to initiate statin therapy. The recommended LDLc therapeutic goal is $<$2.50 mmol/l in high risk individuals (SCORE 5–9%) and $<$1.80 mmol/l or a 50% reduction in LDLc in very high risk individuals (SCORE $\geq$10%) [1].

There is a direct relationship between serum LDLc and incidence of CVD. Similarly, there is a strong inverse association between HDLc and CVD, although recent Mendelian randomization studies found no causal relationship between genetically decreased or increased HDLc and the risk of myocardial infarction [3,4]. However, our concern about including HDLc and LDLc in risk estimation models relates to the potential for analytical error due to imprecision and bias of the lipid measurements. Despite the widespread belief that the calculation or measurement of LDLc or HDLc is standardized and reproducible, results can vary significantly with methods from different manufacturers. In the previous century, the earliest measurements involved ultracentrifugation and precipitation for isolation of LDL and HDL [5]. In the late 1990s, "homogeneous" or "direct" LDLc and HDLc methods have been introduced in the clinical laboratories and largely replaced the older assays [6–8]. Direct LDLc and HDLc methods are commercially available as ready-to-use reagents, enabling full automation of the measurements, however their bias (deviation from "true" value) is a major point of concern. Discrepant results have been reported among the various direct methods, particularly in hypertriglyceridemic and dyslipidemic samples [9–13]. This is also evident from large-scale accuracy-based quality surveys organized across different laboratories [14]. Problems with direct HDLc assays also raise concerns about the reliability of calculated LDLc and non-HDLc treatment goals [12]. Poor reliability of these methods relate to the heterogeneity of both LDL and HDL particles [11,12].

In this study, we aimed to illustrate the potential impact of analytical errors in current LDLc and HDLc measurements on making clinical decisions. A simulation is used here to explore potential CVD risk misclassifications as defined by the SCORE model. Misclassification may occur if a true lipid concentration is within a desirable range, but the reported lipid value is in a high-risk range, or if a true lipid concentration is in a high-risk range but the reported lipid value is in a desirable range [15]. These misclassifications represent a clinically relevant issue because they reflect the practically difficult situation with treatment options: to avoid unnecessary treatment of a patient whose lipid concentration is in a desirable risk category, or failure to treat a patient whose lipid concentration is in a high-risk category, and to distinguish between 'moderate' and 'high-risk' categories when lipid values are near a cutpoint [15]. Misclassification as defined here is of greatest concern because of its potential impact on the patient and healthcare economics. Using data of the Dutch National EQA-2012 external quality assessment of clinical laboratories, representing all LDLc and HDLc reagent systems used in The Netherlands, we simulated the effects of analytical error and hypertriglyceridemia on HDL-adjusted SCORES and concordance of treatment goals.

2. Materials and methods

2.1. Samples

The Dutch external quality assessment (EQA) organizer, the *Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek* (SKML), runs an accuracy-based EQA scheme for clinical chemistry analytes including lipids and apolipoproteins. Quality of the Dutch EQA program has been described previously [16,17]. Briefly, serum pools are prepared in an ISO 13485:2003 certified production facility according to CLSI C37-A protocol [18] and value-assigned for total cholesterol, LDLc and HDLc with CDC Reference Methods in the Lipid Reference Laboratory in Rotterdam, an international member of the CDC Cholesterol Reference Method Laboratory Network (CRMLN). Serum pools are assigned for apolipoprotein B (apoB) at Leiden University Medical Center (LUMC) and are traceable to the WHO-IFCC reference material SP3-08 [19]. The regular EQA-scheme encompasses the analysis of 24 fresh frozen commutable samples per year, i.e., one sample has to be analyzed per two week intervals. About 200 Dutch clinical chemistry laboratories participate in the EQA survey for serum lipids. EQA-test results are electronically submitted through Qbase to the SKML.

In this paper we consider national EQA-results from three normotriglyceridemic (NTG) serum pools (2012.1F, 2012.2D and 2012.2F) and two hypertriglyceridemic (HTG) serum pools (2012.1D and 2012.2E), prepared to study the effect of hypertriglyceridemia. For this purpose, original HTG pools from a selected single donor with triglycerides (TG) $\sim$11 mmol/l were mixed with a NTG serum pool in order to end up with a total TG of $\sim$7 mmol/l. HTG serum pool 2012.1D is a fresh frozen pool stored at -84 °C, whereas 2012.2E is a fresh pool. After aliquoting, NTG and HTG (2012.1D) frozen serum pools were transported to the participating laboratories on dry ice and kept frozen at -84 °C until analysis. Lipid and apolipoprotein measurements, including the target value assignments, of the fresh HTG aliquots (2012.2E) were performed within 2 days upon storage at 4 °C. The participating labs were instructed by SKML to store and process the specimens correctly.

2.2. Biochemical measurements

Direct HDLc measurements were performed in 2012 with state-of-the-art homogeneous methods using Accelerator Selective Detergent (Abbott Diagnostics Division, Beckman Coulter, Siemens Healthcare Diagnostics), PEG-modified enzymatic reagent (Roche Diagnostics), immunoinhibition (Olympus), or Vitros reflectometry slide technology (Ortho Clinical Diagnostics) on automated instruments from the same manufacturers. In 123 laboratories, LDLc was calculated with the Friedewald equation LDLc = cholesterol − HDLc − TG/2.22 (in mmol/l) [20], using direct HDLc from each manufacturer and cholesterol and TG measurements from the same manufacturer in the calculation. In other laboratories ($n = 49$), direct LDLc measurements were performed using α-cyclodextrin sulfate–dextran sulfate Mg^{2+} (Roche) or selective detergent methods (Abbott, Beckman, Ortho, Siemens) on the same instruments as for HDLc. In some but not all laboratories ($n = 38$), apoB was measured with immunonephelometry (Beckman, Siemens) or immunoturbidimetry (Abbott, Roche) instruments. Non-HDLc was not reported by the laboratories but calculated in this study by subtracting HDLc from total cholesterol values in the EQA