Review

Laboratory approaches for predicting and managing the risk of cardiovascular disease: postanalytical opportunities of lipid and lipoprotein testing\textsuperscript{1)}

Michel R. Langlois\textsuperscript{1,2,*}

\textsuperscript{1}Asklepios Core-lab, Department of Laboratory Medicine, AZ St-Jan Hospital Bruges and Ghent University, Ghent, Belgium
\textsuperscript{2}EFCC Working Group Cardiac Markers, European Federation of Clinical Chemistry and Laboratory Medicine

Abstract

Lipoprotein-related risk of cardiovascular disease (CVD) can be adequately predicted in subjects with elevated total cholesterol and low-density lipoprotein (LDL-cholesterol) using the available guidelines. However, individuals with dyslipidemia can have normal total- and LDL-cholesterol concentrations. Many statin-treated patients remain at high residual risk of CVD despite achieving LDL goals. The small dense LDL phenotype, frequently presenting with hypertriglyceridemia and low high-density lipoprotein (HDL-)cholesterol (lipid triad), may contribute to failure to identify and treat high-risk individuals. Therefore, calculated non-HDL-cholesterol is recommended as secondary therapeutic target to LDL-cholesterol in patients with hypertriglyceridemia and mixed dyslipidemia. On-treatment apolipoprotein B adds prognostic information to LDL- and non-HDL-cholesterol by indicating the total number of atherogenic lipoproteins, regardless of their cholesterol content. Risk may be higher than indicated in the risk estimation systems in additional subjects with elevated lipoprotein(a) and homocysteine concentrations. To improve the (post-)analytical phase of lipid tests, aiming for maximal health outcome effectiveness of test interpretation and utilization, laboratory professionals should deliver clinical added value services by providing readily interpreted and guideline-adjusted test reports, interpretative commenting, proactive reflex testing or recommending additional tests, and joining multidisciplinary cooperations in guideline development and cost/benefit studies.

Keywords: cardiovascular disease; clinical added value; lipoproteins; postanalytical phase; risk biomarkers.

Introduction

Given the major public health importance of cardiovascular disease (CVD), guidelines have been developed to assist clinicians and other health professionals in the implementation of health strategies and individual risk measures and targets to promote cardiovascular health and to prevent CVD in day-to-day clinical practice (1–4). Atherosclerosis has a long asymptomatic latent period, which provides an opportunity for early preventive intervention. Guidelines encourage the use of risk scoring systems to facilitate risk estimation in apparently healthy persons with no signs of clinical or pre-clinical disease. In most available scoring systems, the risk for developing CVD is assessed using the multifactorial approach based on age, gender, systolic blood pressure, smoking status, and total cholesterol (TC) concentration (1–4). In patients with dyslipidemia, prevention strategies with either lifestyle changes or lipid-lowering agents (mainly statins) are primarily targeted to low-density lipoprotein-cholesterol (LDL-C). Thus, beyond TC and LDL-C, other lipid tests contribute rather modestly to CVD risk assessment and do not have a major impact in medical decision-making.

The success of preventive interventions is highly dependent on accurate assessment of the individual’s risk. Inaccurate risk assessment leads to failure to identify and treat high-risk people, or to less cost-effective management and overtreatment of those at lower risk. The risk is adequately predicted in subjects with elevated TC and LDL-C, and in patients with known CVD, diabetes, and microalbuminuria/chronic kidney disease (1, 3). However, alarming results emerged from the EUROASPIRE III Study, a survey of 22 European countries (5). High-risk individuals are still not being managed effectively, with too few of these patients following the European guidelines for CVD prevention. Blood pressure, lipid, and glucose control are inadequate, with most patients drastically undertreated and not achieving the targets defined in the guidelines (5). In major epidemiological studies and interventional trials, statin-treated populations with dyslipidemia remain at high residual risk of CVD despite achieving targets for LDL-C, blood pressure, and glycemia, indicating the need for secondary targets of therapy to reduce residual risk (6).
Multidisciplinary efforts can effectively alter management of dyslipidemia and modify risk factors. To improve the post-analytical phase of lipid tests, aiming for maximal health outcome effectiveness of test interpretation and utilization, there is an urgent need for closer interactions between laboratory professionals and clinicians in an integrated approach to: a) appropriate choice and use of risk score; b) methodological aspects of test interpretation; c) other post-analytical aspects including the use of emerging risk factors; and c) cost-effectiveness of comprehensive lipoprotein testing. Opportunities for comprehensive lipoprotein testing and clinical added value services were presented at the EFCC Symposium ‘A scientific approach to the post-analytical phase’ of the IFCC WorldLab-EuromedLab Congress, Berlin, May 18, 2011.

Which risk score has to be used?

Many risk assessment systems are available including Framingham, SCORE (Systemic Coronary Risk Estimation), ASSIGN (from the Scottish Intercollegiate Guidelines Network), Q-RISK, and PROCAM (Prospective Cardiovascular Munster study) (1–4). These scores are intended to combine multiple traditional risk factor measurements into a single quantitative estimate of risk that can be used to target preventive interventions. In practice, most risk estimation systems can be applied to populations similar to that from which the system was derived, but need to be recalibrated for use in different populations (4).

Most but not all scores include HDL-cholesterol (HDL-C) as additional risk-modifying factor and carefully differentiate between men and women. Diabetes mellitus is not included in the Framingham and SCORE risk models, but the guidelines designate diabetes as the highest risk equivalent and recommend that it should be treated as a very high-risk condition (1–3). Unlike Framingham and SCORE, PROCAM includes family history of myocardial infarction, triglycerides (TG), LDL-C, and diabetes as risk factors. However, PROCAM can only be used in men under the age of 65 years; it is derived from a prospective study of a male cohort in the German city of Münster. ASSIGN (developed in Scotland) and QRISK (UK) incorporate socio-economic deprivation and family history in the risk score; the QRISK model also includes ethnicity and body mass index (4).

The current joint European Task Force on CVD prevention in clinical practice clearly recommends the use of the SCORE system because it is based on large, representative data sets from 12 European cohort studies including 205,178 patients and 7934 CVD deaths, and it has been externally validated in separate, independent cohorts (1). Unlike Framingham and other models that calculate global risk of fatal and non-fatal CVD, the SCORE system estimates the 10-year risk of fatal CVD only (1). It allows the use of risk charts that can be recalibrated according to time-trends in CVD mortality in the different low-risk or high-risk countries. The use of the low-risk chart is recommended in Belgium, France, Greece, Italy, Luxembourg, Spain, Switzerland and Portugal; the high-risk chart should be used in all other countries for Europe (1). Several countries including Belgium, Germany, Greece, The Netherlands, Poland, Spain and Sweden have undertaken national recalibrations of the SCORE charts using local national mortality statistics and prevalence rates for major risk factors. Intervention strategies are proposed as a function of SCORE (very high-risk: ≥10%; high-risk: ≥5% and <10%; moderate risk: ≥1% and <5%; low risk: <1%) and LDL-C (3).

Limitations of conventional lipid tests

LDL-cholesterol

Individuals can have exactly the same LDL-C concentration, but might differ significantly in CVD risk. The existence of physicochemically heterogeneous subclasses of LDL may contribute to this observation. “LDL” comprises a heterogeneous group of particles (subclasses) that differ in size, density, chemical composition and in their associations with atherosclerosis. Two distinct LDL subclass phenotypes have been assigned as phenotype A, with a lipoprotein profile consisting primarily of the larger buoyant LDL subfractions, and phenotype B, with predominantly smaller and denser LDL subfractions (7). Prospective studies have demonstrated an association between phenotype B and the risk of CVD, although not always independently in multivariate analyses (7–10). Small dense LDL (sd-LDL) particles easily penetrate into the subendothelial space of the arterial wall and are highly atherogenic, particularly if they are oxidatively modified (11). A person with normal TC and LDL-C but with predominantly sd-LDL might be at high risk, although this may not be apparent from the risk assessment score. However, the additional benefit of measuring LDL particle size and number above LDL-C testing to identify high-risk persons remains to be confirmed.

HDL-cholesterol

The risk of coronary heart disease increases 2%–4% for every 1 mg/dL decrease of HDL-C (12). This strong and independent association persists in statin-treated patients (12). Of particular concern for the interpretation of HDL-C values, however, is the heterogeneity of the lipid fraction referred to as “HDL” (12). The HDL fraction comprises a family of particles that differ in density, size, shape, lipid and apolipoprotein composition, or in association with CVD risk. Classically, three main subclasses preβ-HDL, HDL2 and HDL3 are recognized, but specialized analytical techniques have demonstrated many more subpopulations (12).

Both the quantity and the quality of HDLs are important with regard to CVD risk. Large HDL2 particles are thought to be more atheroprotective than small HDL3 particles (12). With moderately increasing triglyceridemia, cholesterol esters are preferentially transferred from HDL2 to very low-density lipoproteins (VLDL) (13). This results in the formation of sd-LDL, a decreased HDL2/HDL3 ratio, and decreased HDL-C (13). The coexistence of a small dense LDL phenotype,
together with low HDL-C and elevated TG, is referred to as the “atherogenic lipoprotein phenotype” (lipid triad) that is very common in patients having a high risk for early-onset atherosclerotic disease. Guidelines propose to consider adding fibrate or nicotinic acid to LDL-lowering therapy in high-risk patients with a high TG/low HDL-C phenotype or with isolated low HDL (14).

**Triglycerides and metabolic syndrome**

Although the role of TG as a causative, independent marker of CVD risk has been strongly debated and remains controversial, recent data strongly favor the role of TG-rich lipoproteins as a risk factor for CVD (15–17). The risk is associated more strongly with moderate than with severe hypertriglyceridemia (≥500 mg/dL), probably because the latter is often due to large VLDLs and chylomicrons that are not atherogenic but can cause pancreatitis (18). Mild-to-moderate hypertriglyceridemia is strongly associated with type 2 diabetes and the metabolic syndrome (MetS) that promote the development of early-onset CVD (18). MetS describes the clustering of cardiovascular risk factors – hypertension, low HDL-C, hypertriglyceridemia, impaired fasting glycemia – together with central (abdominal) obesity and hyperinsulinemia/insulin resistance (19). It identifies individuals with increased risk of developing CVD and type 2 diabetes. This implies that, if one component is identified, a systematic search for the others is indicated, together with an active approach to managing all these risk factors. Hypertriglyceridemia combined with low HDL-C and a preponderance of sd-LDL particles (lipid triad) is often seen without elevated TC or LDL-C. Other – less specific – features of MetS are hyperuricemia, microalbuminuria, and a pro-inflammatory state (19).

The diagnosis of MetS may identify additional patients with high risk of CVD among those who would be classified in a lower risk category using the conventional risk charts. Any person with obesity, elevated TG, low HDL-C, or MetS is a candidate for therapeutic lifestyle changes to modify these risk factors regardless of LDL-C concentration (3, 14, 20).

Measurement of TG is traditionally performed after extended (12 h)-fasting. It has been proposed, however, that postprandial creation of TG remnant particles is an important factor in atherogenesis (21). Non-fasting TG concentrations may therefore be more relevant to the estimation of CVD risk than fasting TG. At present, this approach is restricted by the practical disadvantage that there is no accepted cutpoint of non-fasting TG that clearly identifies increased risk.

**Methodological limitations**

LDL-C can be measured directly with homogeneous assays, but is usually calculated by the Friedewald formula. Like direct LDL-C measurements, the LDL-C calculation is not without errors in dyslipidemic subjects (22, 23). The Friedewald formula also includes the intermediate-density lipoprotein (IDL) and Lp(a) cholesterol components and makes assumption of a standard triglycerides:cholesterol ratio in VLDL, a lack of chylomicrons, and a lack of excessive remnant lipoproteins. The equation is accurate in samples with TG <200 mg/dL, but the calculation is invalid when TG concentrations are >400 mg/dL or in the case of type III dyslipoproteinemia (VLDL remnants) (1). That is because the triglyceride:cholesterol ratio in TG-carrying lipoproteins (VLDL and chylomicrons) progressively increases as hypertriglyceridemia becomes more severe, and the equation would overestimate VLDL-cholesterol and therefore underestimate LDL-C (1). The fact that the calculation depends upon three laboratory measures means that three coefficients of variation are involved with potential for error. Hypertriglyceridemia >200 mg/dL and inaccurate HDL-C measurements might cause post-analytical errors in CVD risk classification and treatment options based on calculated LDL-C (23). The risk associations of HDL-C and LDL-C are opposite and the error is reciprocal; erroneously increased HDL-C (positive bias) leads to falsely decreased LDL-C and risk is underestimated twice.

The heterogeneity of HDLs represents a major challenge in the measurement of HDL-C. The innovative homogeneous HDL methods enable direct, precise, and fully-automated HDL measurement but their accuracy (estimation of “true” value) is a major point of concern. Discrepant results between direct methods and early precipitation methods, and between various direct methods, are attributable to differing detection of specific HDL subpopulations that may or may not be included, to co-detection of non-HDL particles, or to interferences of high TG, dyslipoproteinemias, and monoclonal para-proteins (12). The Centers for Disease Control and Prevention (CDC) Reference Method combines ultracentrifugation with precipitation to separate and measure HDL. However, considering the heterogeneity of HDL particles, there is no evidence that the particular “HDL” fraction obtained by the CDC Reference Method is a better indicator of CVD risk than the fractions obtained by any other method (12).

**Novel biomarkers**

In recent years, the number of new candidate risk factors proposed as predictors of CVD and its complications has grown considerably (24). Both residual risk for patients already taking statins and the increasing prevalence of obesity, which has shifted the risk profile of the population towards patients in whom LDL-C is less predictive of CVD, have contributed to increasing interest in the new markers. These include lipoprotein subclasses and particle concentration, Lp(a), apolipoproteins (apo)A-I and apoB, inflammation markers, homocysteine, and markers of renal function. These biomarkers are termed “emerging risk factors” because they are associated with an increased risk for CVD, but their causative, independent, and quantitative contributions to CVD are not as well documented as the major, longest established risk factors – hypercholesterolemia, hypertension, and smoking (24). An emerging marker may not be necessarily a newly discovered marker, but may be an existing marker or a calculated marker, e.g., non-HDL-cholesterol (non-HDL-C). Only few
data show additional benefit over the classical risk factors in well-done prospective studies and multivariate analysis. None of the emerging markers should be used as screening tests, and methodological problems, such as preanalytical factors and lack of standardization complicate the use of most emerging markers in daily clinical practice (24). Among the many emerging risk markers, only non-HDL-C and apoB independently seem to add incremental value beyond traditional LDL-C, and guidelines are now available establishing apoB as effective for identifying residual risk or for monitoring treatment.

**Non-HDL-cholesterol**

In the fasting state, non-HDL-C represents the cholesterol in atherogenic particles LDL, IDL, VLDL, and Lp(a). Calculated by simply subtracting HDL-C from TC, non-HDL-C is a useful alternative to calculated LDL-C for patients with TG >400 mg/dL (4.5 mmol/L) wherein the Friedewald equation is invalid.

Non-HDL-C provides an even better risk estimation than does LDL-C, in particular in hypertriglyceridemia combined with diabetes, MetS, or chronic kidney disease, and there is a direct, consistent relationship between the magnitude of non-HDL-C lowering and CVD risk reduction (25). Guidelines recommend that non-HDL-C should be monitored as a secondary target of therapy when the primary goal for LDL-C has been achieved but TGs remain high, >200 mg/dL (2.26 mmol/L), in order to take into account the atherogenic potential of remnant lipoproteins (3, 14). For those who have lower TG, LDL-C is still considered a sufficient target alone (3, 14). The recommended cutpoints for non-HDL-C are arbitrarily set 30 mg/dL higher than LDL cutpoints because the VLDL cholesterol associated with the TG cutpoint of 150 mg/dL is 30 mg/dL (=TG/5). Thus, a non-HDL-C goal of <130 mg/dL is equivalent to the LDL-C goal of <100 mg/dL in high-risk subjects (Table 1).

Risk is more directly related to the number of atherogenic lipoproteins than to the cholesterol content of the lipoproteins (26, 27). Non-HDL-C, like LDL-C, does not reflect particle number and therefore could underestimate risk in subjects with small cholesterol-depleted LDL particles (phenotype B). Furthermore, inaccurate HDL-C measurements with homogeneous assays might contribute to inappropriate treatment decisions based on non-HDL-C. However, non-HDL-C is a cost-effective alternative to LDL-C as the calculation incurs no additional expense above conventional lipid testing, can be obtained in the non-fasting state, and is treatable with existing lipid-lowering agents (3).

**Apolipoproteins B and A-I**

ApoB is the major protein component of LDL, IDL, and VLDL. Since there is one apoB molecule per particle, concentrations of apoB are therefore a direct measure of the total number of atherogenic lipoproteins in plasma, regardless of their size (26, 27). Clearly apoB is currently the best emerging marker and is superior, or at least equal, to traditional lipid measures to predict CVD risk (28–34). Elevated apoB concentrations can help to identify additional high-risk subjects with a small, dense LDL phenotype B (normocholesterolemic hyper-apoB) who were categorized as “moderate risk” at baseline scoring.

ApoB has been more extensively validated in epidemiological studies and clinical trials than non-HDL-C (Table 2). On-treatment apoB adds prognostic information to LDL-C and even to non-HDL-C in both primary- and secondary-prevention trials (35–41). ApoB is superior to LDL-C to judge adequacy of lipid-lowering therapy, particularly in patients with low or normal LDL-C, and to suspect high residual risk in treated patients who have achieved their LDL-C goal. The guidelines propose apoB as alternative to non-HDL-C as a secondary target for monitoring lipid-lowering therapies in patients with hypertriglyceridemia (Table 1) (3, 24, 42).

ApoA-I is the major protein component of HDLs and plays a central role in cholesterol efflux from the cells (12). Its measurement reflects both the quantity (number) and quality of HDL particles, because the dense HDL3 subfraction contains less apoA-I molecules than large, mature HDL2 particles. Furthermore, inflammation-sensitive plasma proteins, such as serum amyloid A (SAA) displace apoA-I from HDL, resulting in the formation of pro-atherogenic HDL particles and a decrease in the proportion of apoA-I among HDLs (43). In most epidemiological studies, however, apoA-I measurements do not provide incremental value over HDL-C to predict CVD risk. The apoB/apoA-I ratio can be used as an alternative to the usual TC/HDL-C ratio to determine lipoprotein-related risk (24).

From a methodological point-of-view, apoB and apoA-I are the best risk markers because the assays are more accurate than traditional LDL and HDL assessments, internationally standardized, and easily automated (27). A practical disadvantage, however, is that measurements of apoB and apoA-I are not widely performed in clinical laboratories due to inconsistent reimbursement policies by the national governments across different countries, and thus not generally available to all clinicians.

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**Table 1**  
Primary and secondary targets of preventive therapy according to CVD risk categories assessed with the SCORE system.

<table>
<thead>
<tr>
<th>Risk</th>
<th>LDL-C, mg/dL (mmol/L)</th>
<th>Non-HDL-C, mg/dL (mmol/L)</th>
<th>ApoB, mg/dL (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high</td>
<td>&lt;70 (1.8)</td>
<td>&lt;100 (2.6)</td>
<td>&lt;80 (0.8)</td>
</tr>
<tr>
<td>High</td>
<td>&lt;100 (2.5)</td>
<td>&lt;130 (3.3)</td>
<td>&lt;100 (1.0)</td>
</tr>
<tr>
<td>Moderate</td>
<td>&lt;115 (3.0)</td>
<td>&lt;145 (3.8)</td>
<td>&lt;120 (1.2)</td>
</tr>
</tbody>
</table>

CVD, cardiovascular disease; SCORE, Systematic COronary Risk Evaluation. ‘10-year risk of CVD death. Very high risk: ≥10% or documented CVD, type 2 diabetes, type 1 diabetes with microalbuminuria, chronic kidney disease. High risk: ≥5% and <10% or familial dyslipidemia, severe hypertension. Moderate risk: ≥1% and <5% (3). Primary target in cases of hypertriglyceridemia >400 mg/dL (invalid LDL-C calculation); secondary target in patients with combined dyslipidemia, metabolic syndrome, type 2 diabetes, or chronic kidney disease.
Table 2  Major studies on the benefit of apoB and apoB/A-I ratio over traditional lipids for predicting CVD risk.

<table>
<thead>
<tr>
<th>Studies and key conclusion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiological studies</strong></td>
<td></td>
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<tr>
<td>AMORIS (n=175,553); 6-year prospective follow-up.</td>
<td>(28)</td>
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<tr>
<td>ApoB/A-I is strongly related to risk of fatal myocardial infarction after adjustment for age, TC, and TG; ApoB is a better predictor than LDL-C at low LDL-C concentrations.</td>
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<tr>
<td>INTERHEART (n=15,152 cases, 14,820 controls); case-control study in 52 countries.</td>
<td>(29)</td>
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<tr>
<td>ApoB/A-I is stronger predictor of myocardial infarction than non-lipid risk factors.</td>
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<tr>
<td>MONICA/KORA Augsburg Cohort Study (n=2850); 10-year prospective follow-up study</td>
<td>(30)</td>
</tr>
<tr>
<td>ApoB/A-I and TC/HDL-C have similar discriminant performance to predict fatal and non-fatal coronary events.</td>
<td></td>
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<tr>
<td>Health Professionals Follow-up Study (n=18,225 men); 6-year follow-up study.</td>
<td>(31)</td>
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<tr>
<td>ApoB adds significant prognostic information to non-HDL-C for fatal and non-fatal ischemic heart disease prediction.</td>
<td></td>
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<tr>
<td>Women’s Health Study (n=15,632 women); 10-year prospective follow-up study.</td>
<td>(32)</td>
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<tr>
<td>ApoB and non-HDL-C are better predictors than TC or LDL-C for first CVD events; TC/HDL-C ratio shows the strongest association.</td>
<td></td>
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<tr>
<td>Framingham Offspring Study (n=3322); prospective study (median 15 years follow-up).</td>
<td>(33)</td>
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<tr>
<td>ApoB/A-I does not provide incremental utility over TC/HDL-C for predicting coronary events or death.</td>
<td></td>
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<tr>
<td>ISIS (n=3510 cases, 9805 controls); case-control study.</td>
<td>(34)</td>
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<tr>
<td>ApoB/A-I is more informative about risk of acute myocardial infarction than LDL-C/HDL-C, TC/HDL-C, non-HDL-C, and TC.</td>
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<tr>
<td><strong>Interventional trials</strong></td>
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<tr>
<td>FATS (n=146 men)-secondary prevention trial (lovastatin-colestipol, niacin-colestipol).</td>
<td>(35)</td>
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<tr>
<td>1st interventional trial selecting patients with high apoB (≥1.25 g/L).</td>
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<tr>
<td>Percentage reduction in apoB independently predicts angiographic benefit (change in coronary stenosis).</td>
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<tr>
<td>AFCAPS/TexCAPS (n=6605); primary prevention study (lovastatin).</td>
<td>(36)</td>
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<tr>
<td>On-treatment apoB and apoB/A-I are predictive of risk for first acute major coronary event or death; on-treatment LDL-C is not.</td>
<td></td>
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<tr>
<td>LIPID (n=9014); secondary prevention study (pravastatin).</td>
<td>(37)</td>
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<tr>
<td>On-treatment apoB is better predictor than LDL-C to explain non-fatal or fatal coronary event reduction.</td>
<td></td>
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<tr>
<td>TNT (n=10,001), IDEAL (n=8888); secondary prevention studies (atorvastatin, simvastatin).</td>
<td>(38)</td>
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<tr>
<td>On-treatment non-HDL-C and apoB are more closely associated with CVD outcome than LDL-C.</td>
<td></td>
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<tr>
<td>PROVE-IT TIMI 22 (n=4162); secondary prevention study (atorvastatin vs. pravastatin).</td>
<td>(39)</td>
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<tr>
<td>On-treatment non-HDL-C and apoB offer similar prognostic information to LDL-C for acute coronary events or death.</td>
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<tr>
<td>CARDS (n=2838); primary prevention trial in type 2 diabetes (atorvastatin).</td>
<td>(40)</td>
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<tr>
<td>Statins lower LDL-C (−40%) more than apoB (−24%). Many patients who achieve their LDL-C or non-HDL-C target remain above the recommended target for apoB.</td>
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<tr>
<td>FIELD (n=9795); primary prevention trial in type 2 diabetes (fenofibrate).</td>
<td>(41)</td>
</tr>
<tr>
<td>ApoB and ApoB/A-I are as strong as lipid ratios non-HDL-C/HDL-C, TC/HDL-C, and LDL-C/HDL-C to predict fatal and non-fatal CVD in type 2 diabetics.</td>
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</table>

**Inflammation markers**

There is abundant experimental and epidemiological evidence that circulating markers of activated inflammation are closely associated with the development of atherosclerosis and CVD (44). Cytokine- and adipokine-mediated inflammation has also been linked to central (abdominal) obesity and MetS (45). Some studies have demonstrated that C-reactive protein, measured by high-sensitive immunoassays (hs-CRP), adds prognostic information to LDL-C in risk models. For most individuals, hs-CRP does not add substantial predictive value beyond that provided by conventional risk factors. However, individuals with normal LDL-C who have CRP >3 mg/L might represent a high-risk group often missed on the basis of LDL alone (44). A working group of the American Heart Association and CDC proposed hs-CRP testing as an option in intermediate-risk patients with normal LDL-C (wherein uncertainty remains as to the use of preventive therapies) and in those with MetS; patients with elevated hs-CRP should then be given a higher risk category in the Framingham model (24, 44). The Reynolds Risk Score incorporates hs-CRP in the risk estimation model (46, 47).
The JUPITER trial results demonstrated that statin treatment in healthy individuals who have normal LDL-C but who do have elevated hs-CRP, yielded a marked reduction in risk of CVD events or stroke (48). Statins appear to lower hs-CRP in a cholesterol-independent way. However, there is insufficient data that therapeutic monitoring using hs-CRP over time is useful to evaluate effects of statin treatment in primary prevention.

Despite promising data obtained in large study cohorts, the benefit of hs-CRP testing for the individual patient remains controversial because of preanalytical problems (biological variability) and lack of consistent guidelines. CRP (as well as fibrinogen, SAA, and other inflammation markers) is often seriously confounded by lifestyle variables and pre-clinical disease causing rise in CRP (49), so overall utility is daily practice is questionable. Another inflammatory marker, lipoprotein-associated phospholipase A2 (Lp-PLA2), is less variable than CRP and related more specifically to vascular inflammation and rupture-prone plaque (50, 51). Although the measurement of Lp-PLA2 mass or activity appears a promising biomarker for identifying “hidden” high-risk patients (50, 51), concrete recommendations may be premature because clinical validation studies need to be completed.

Lipoprotein(a)

Lp(a) is a cholesterol-rich LDL particle with one molecule of apoB to which is attached an additional protein, apolipoprotein(a), via a disulfide bond (52). Apolipoprotein(a) contains 10 different types of kringle 4-like repeats that are structurally homologous to plasminogen. The kringle 4 type 2 domain is present in multiple repeated copies from two to >40 that differ in number between apolipoprotein(a) isoforms (52).

High Lp(a) concentrations are strongly inherited and they identify persons having a genetic predisposition of CVD (24), particularly when LDL-C and Lp(a) are concomitantly elevated. The strong synergistic effect between LDL-C and Lp(a) points to a benefit of Lp(a) testing in intermediate-risk patients with moderately elevated LDL-C. Risk may be higher than indicated in the risk score in subjects with elevated Lp(a) (1). It is recommended that Lp(a) should be measured in all subjects with premature CVD (men <55 years, women <60 years) and in subjects at moderate risk with a family history of premature CVD and/or elevated Lp(a), or recurrent CVD despite statin treatment (52); patients with elevated Lp(a) should then be considered to be at higher risk than initially scored and need more intensive LDL-lowering therapy.

Lp(a) should be measured once, using an apolipoprotein(a) size-insensitive assay; repeat measurement is only necessary if treatment for high Lp(a) is initiated in order to evaluate therapeutic response (52). As a secondary priority after LDL-C reduction, a desirable level for Lp(a) <50 mg/dL has been recommended but treatment to reduce Lp(a) with nicotinic acid remains controversial (52).

Homocysteine

Measurement of homocysteine in the general population to screen for CVD risk is not recommended (24). In young CVD patients (<40 years), homocysteine should be measured to exclude inborn errors of metabolism (“homocystinuria”) that may cause very high plasma concentrations (>100 μmol/L) (53). A recent study suggested a benefit of homocysteine testing in intermediate-risk persons to improve risk prediction with the Framingham model (54). Intermediate-risk patients with hyperhomocysteinemia (>15 μmol/L) may be at higher risk than indicated in the risk estimation model, and they should receive further laboratory assessments and optimal treatments for known causal risk factors (folate or vitamin B12 deficiency, impaired renal function) (53). However, large-scale intervention trials showed no significant beneficial effect of treating mild hyperhomocysteinemia with folate and B vitamins to reduce risk of CVD or all-cause mortality, despite adequate homocysteine lowering (55).

(Post-)post-analytical laboratory approaches

The clinical laboratory plays a key role in the cardiovascular risk assessment of patients with dyslipidemia. Clinicians that are involved in CVD prevention are highly dependent on accurate in vitro diagnostic information. Incorrect diagnosis and mismanagement of treatment, which are based on laboratory measures, are both costly to society and harmful to the patient. Beyond the importance of analytical and operational excellence in lipid testing, laboratory professionals should enhance their role and engagement in the (post-)post-analytical phase by creating clinical added value services (more than data production).

Clinical added value and consultative support to clinicians may be created by implementing reflex testing and diagnostic algorithms, interpretative commenting, and guiding therapeutic options related to lipid tests. Post-analytical efforts are needed not only on the local laboratory level, but also on the (inter-)national level by taking responsibility for successful implementation of the appropriate guidelines in the regional laboratories, harmonizing lipid profiles, and initiating cost/benefit studies aiming to help the clinicians to improve CVD risk management (the patient’s perspective) and reduce healthcare costs (the society’s perspective) (Table 3). Laboratory services should be delivered as part of an integrated healthcare system, combining operational excellence and a medical knowledge network to seek maximal outcome effectiveness in CVD prevention (56). The key is to understand the information that is provided by the laboratory test in a given clinical setting, evaluate in the context of the patient’s unique situation, and integrate these data to make a CVD risk estimation and therapeutic decision.

Test reporting

Laboratory test reporting is the post-analytical opportunity to guide clinicians in cardiovascular risk assessment and
on management of their patients. Successful implementation of the guidelines will require that laboratories provide accurate lipid profiles to the clinician in an appropriate and readily interpreted report format (57). Laboratories need to adjust reporting formats and interpretations, continuously updating of risk cutpoints and target values according to the appropriate guideline applicable for the population for which the risk assessment model can be used. Report formats should consistently provide the target values, not “reference ranges”, of the lipid measurements. Calculated parameters, such as non-HDL-C and TC/HDL-C ratio should be automatically included on laboratory report forms whenever TC and HDL-C are measured, and apoB/A-I ratio when both apolipoproteins are measured. Even better, laboratories could report non-HDL-C as a reflex value only for those patients with TGs ≥200 mg/dL (2.26 mmol/L) as recommended in the guidelines (57).

**Interpretative commenting**

Interpretative comments are useful for delivering diagnostic information. In contrast to the relatively easy interpretation and risk management of hypercholesterolemia and elevated LDL-C (type IIa hyperlipidemia), guidance in test interpretation can add clinical value to the lipid profiles with mixed dyslipidemias and hypertriglyceridemias (type IIB or IV).

Suggesting to rule-out MetS can draw the clinician’s attention to this disorder in lipid profiles with low HDL-C (<40 mg/dL in men, <50 mg/dL in women), high TG (≥150 mg/dL, and impaired fasting glycemia (≥100 mg/dL). Interpretative commenting the level of hypertriglyceridemia (“mild”, “moderate”, or “severe”) is useful because it determines the diagnostic and therapeutic management options (57, 58).

- When TG are borderline high, 150–199 mg/dL (1.7–2.2 mmol/L) (“mild hypertriglyceridemia”), emphasis should be upon lifestyle modifications.
- In “moderate hypertriglyceridemia”, 200–499 mg/dL (2.3–5.6 mmol/L), non-HDL-C or apoB become the secondary target of therapy after LDL-C. In addition to lifestyle modifications, drug therapy should be considered to achieve the non-HDL-C or apoB goal in high-risk patients, by initiating or intensifying statin therapy or by adding omega-3-fatty acids, nicotinic acid, or (in selected patients under strict control of side effects) fibrates (3).
- In “severe hypertriglyceridemia” ≥500 mg/dL (5.7 mmol/L) the initial goal is to prevent pancreatitis by lowering TG (3, 58). Patients with TG>1000 mg/dL (11.3 mmol/L) may have lipoprotein lipase (LPL) defect; the use of fish oil and omega-3-fatty acids is contraindicated in patients with severe LPL deficiency (chylomicronemia syndrome).

Recommending repeat testing of lipids and hs-CRP can assist the clinician to account for biological variability. Laboratories should recommend to take at least two measures of hs-CRP, 2 weeks apart, with the lowest value being used to estimate CVD risk, a practice consistent with recommendations for lipid evaluation (24). However, considering the
large within-subject biovariability of CRP (49), more sequential measures might be necessary in certain patients and, in my opinion, systematic hs-CRP testing should be discouraged. This may be considered at levels of moderate risk just below the 5% high-risk threshold; hs-CRP >3 mg/L may then help to motivate drug intervention in older men >50 years and women >60 years with MetS and/or uncertain adherence to lifestyle intervention (2). CRP concentrations >10 mg/L, indicating acute inflammatory or infectious disease, should not be used and the test should be repeated after 2 weeks when the patient is clinically stable (24).

Recommending family screening and childhood screening for familial hypercholesterolemia (FH) is useful in cases of very high LDL-C >200 mg/dL (>135 mg/dL in children) (3, 59). This may not be necessary in hospital laboratories supporting specialized lipid clinics, but may be helpful to assist general practitioners in their preventive strategies. Close relatives of patients with premature CVD and children who belong to families with inherited dyslipidemias, such as FH are at increased risk of developing CVD and should be examined for all risk factors including apoB and Lp(a) (52).

**Additional testing**

Information technology-supported decision systems and real-time electronic ordering (decentralized order entry) can assist clinicians in rule-driven generation of tests orders. This can be anticipated in the post-analytical phase by proactive reflex testing or at least recommending additional test orders, e.g., apoB measurement in cases of invalid LDL-C calculation (TG >400 mg/dL) or suspected LDL subclass phenotype B (in a lipid profile with high TG and low HDL).

ApoB testing adds diagnostic and prognostic information by separating higher and lower risk patients with mild-to-moderate hypertriglyceridemia. Familial combined hyperlipidemia (FCHL) and familial hypothyroidalphoproteinemia are the most common and probably underdiagnosed lipoprotein disorders: each affect about 1% of the general population and are most accurately diagnosed with a lipid panel that includes apoB and TG (3, 18, 27). Each of these disorders shares features with type 2 diabetes and MetS and warrant aggressive interventions to reduce the risk of (premature) CVD. In contrast, one other inherited form of hypertriglyceridemia—monogenic familial hypertriglyceridemia—is not associated with premature CVD; this disorder also affects up to 1% of the population and must be distinguished from FCHL in making treatment decisions (18, 58).

- The evaluation of mild-to-moderate hypertriglyceridemia should initially focus on whether there is a family history of hypertriglyceridemia or a personal or family history of premature CVD; in addition, potential secondary causes should be identified (18, 58).
- Patients with FCHL may have elevated or normal LDL-C and present with phenotypes IIa, IIb or IV (60). Low HDL-C and increased sd-LDL particle number (and therefore apoB) are present in both FCHL and familial hypothyroidalphoproteinemia. In patients with mild-to-moderate hypertriglyceridemia who do not have clinical premature CVD, or in those with unreliable family history of premature CVD, the measurement of apoB helps to distinguish FCHL from the less atherogenic familial hypertriglyceridemia without increased sd-LDL particle concentration; apoB concentrations are elevated (>1.2 g/L) in FCHL adults and children but are low or normal in familial hypertriglyceridemia (18, 60).
- Apo-A-I measurement helps to distinguish FCHL from familial or primary causes of hyperalphaloproteinemia in patients with a high TG/low HDL profile (18); low apoA-I concentration (<1.1 g/L in men, <1.2 g/L in women) is mostly found in hyperalphaloproteinemias.

A nomogram for the diagnosis of FCHL has been published (61). Lacking a specific laboratory marker, the final diagnosis of FCHL is difficult in patients with features of MetS. The main differences between the two conditions could orientate the differential diagnosis but do not prove it (62):

- ApoB is constantly high in FCHL, but not in MetS. LDL-C concentrations are usually normal or rather low in MetS.
- The inheritance of the disorder is much more evident, and clinical and laboratory manifestations are earlier in FCHL than in MetS.
- Low-grade inflammation (e.g., elevated hs-CRP, plasma fibrinogen) and/or hyperuricemia are more frequent in MetS.

For ruling-out secondary causes of dyslipidemias, additional tests for creatinine, glucose, thyrotropin, cortisol, bilirubin, alkaline phosphatase, albumin, and recommending a urinalysis for proteinuria (microalbuminuria) are all useful (63). Patients with fasting TG>2000 mg/dL (22.6 mmol/L) almost always have both a secondary and a genetic form of hypertriglyceridemia (18). Hyperlipidemic myeloma is a rare variant of myeloma in older patients (>55 years) presenting with skin xanthomas and/or hyperviscosity syndrome (64). Monoclonal paraproteins may have a direct inhibitory effect on lipoprotein clearance in vivo but may also cause analytical interference resulting in falsely low or undetectable HDL-C with direct methods (12). Serum protein electrophoresis has a specificity of approximately 92% and a sensitivity of approximately 80% to identify paraproteins; specificity and sensitivity increase to >97% by combining protein electrophoresis with immunofixation and/or free light chain assays (65).

**Standardizing risk assessment**

To aim for total quality of the testing process, laboratories could take full responsibility in risk assessment by calculating and reporting the risk scores with the lipid profiles (when the patient’s clinical data and non-lipid risk factors are accessible to the laboratory information system), similarly to the laboratory approach to prenatal screening for risk of Down’s syndrome by integrating biochemical and ultrasound data. This should allow more standardized risk assessment, using the appropriate risk scoring tool that is recommended as most relevant to the regional population according to the guidelines. This approach may not be feasible in laboratories that...
have difficulties in collecting clinical data. The integrated approach requires electronic patient data management and tracking, enabling computerized risk scoring. Alternatively, the patient’s clinical data can be provided with electronic test ordering by the clinician. The electronic version of SCORE, HeartScore, is available (www.heartscore.org) for risk calculation (3). With this approach, standardized risk assessment can be achieved in a medical knowledge network of laboratory professionals and clinicians who are jointly responsible for a well-designed CVD prevention program (Table 4).

The score systems are developed for combining all individual risk factor measurements into a single estimate of risk that can be used to target preventive interventions. However, the ultimate risk judgment and management should remain the role of the clinician. The score must be seen as an aid to clinicians in planning preventive strategies with their patients, managing total risk rather than focusing on individual risk factors. Risk scores must be interpreted in the light of the clinician’s detailed knowledge of his patient’s lifestyle, family history, socio-economic status, psychosocial risk factors, compliance to drug therapy and adherence to lifestyle modifications. In this approach, the clinician must evaluate the calculated score in the context of the patient’s unique situation in which deviation from the guidelines may be appropriate, and then raise or lower the assigned risk category after evaluating the presence or absence of any underlying risk factor, such as unhealthy lifestyle and obesity, and make a therapeutic decision. Conversely, risk may be lower than indicated in those with a family history of longevity. The decision to change a patient’s risk category on the basis of his or her underlying or emerging risk factors [apoB, Lp(a), or homocysteine] must be based on clinical judgment, this is beyond the responsibility

### Table 4 Stepwise integrated approach to CVD risk estimation through interaction between laboratory professionals and clinicians in a medical knowledge network using SCORE (3).

1. **Initial tests:**
   - TC
   - TG
   - HDL-C (measured or calculated)
   - Glucose
   Take average of ≥2 fasting measurements with an interval of 1–2 weeks (12 weeks after acute major illness, surgery or trauma).

2. **Estimate 10-year risk of fatal CVD**
   - Calculate SCORE using age, gender, TC, systolic blood pressure, smoking status.
   - Very high-risk:
     - Documented CVD, type 2 diabetes, type 1 diabetes with target organ damage/microalbuminuria, chronic kidney disease.
     - SCORE ≥10%.
   - High-risk:
     - Familial dyslipidemia, severe hypertension.
     - SCORE ≥5% and <10%.
   - Moderate risk:
     - SCORE ≥1% and <5%.
     - This risk can be further modified by HDL-C.
   - Low risk:
     - SCORE <1%.

3. **Additional tests:**
   - Tests to rule-out secondary causes of dyslipidemia.
   - In moderate-risk patients with (unreliable) family history of premature CVD:
     - Lp(a)
     - Homocysteine
   - In mild-to-moderate hypertriglyceridemia with (unreliable) family history of premature CVD:
     - ApoB (>1.2 g/L: probably FCHL)

4. **Adjustment of risk category**
   - Consider higher risk in moderate-risk patients with a family history of premature CVD, abdominal obesity, sedentary lifestyle, elevated apoB, Lp(a), hyperhomocysteinemia.

5. **Interventional strategies**
   - Lifestyle modifications at all levels of risk.
     - Very high-risk: immediate drug intervention if LDL-C ≥70 mg/dL.
     - High-risk: immediate drug intervention if LDL-C ≥100 mg/dL.
     - Moderate risk: consider drug intervention if uncontrolled persistent LDL-C ≥115 mg/dL.
     - Low risk: consider drug intervention if uncontrolled LDL-C >190 mg/dL.
     - Calculate non-HDL-C (treatment target) when TG ≥200 mg/dL or invalid LDL-C calculation.

FCHL, familial combined hyperlipidemia; SCORE, Systematic COronary Risk Evaluation. *Actions/decisions to be taken exclusively by the clinician, with consultative support of the laboratory.
of the laboratory. The problem is that many patients are inadequately treated because of improper decisions made by health professionals; this is the main reason why guidelines are developed and, in my opinion, why laboratories should pro-actively support the implementation of the appropriate scoring tool and close the gap between the guidelines and daily clinical practice.

Cost/benefit studies

A more effective integration of information technology could allow the implementation of quality indicators that can serve as a tool to monitor and control the total testing process, including the (post-)post-analytical activities, such as interpretation and utilization of test information and effects on patient outcome (reduction or prevention of CVD events). The true evaluation of a diagnostic test, beyond its analytical validity, is to demonstrate its effectiveness in helping the clinician achieve a correct CVD risk estimation or improve patient outcome, and its cost-effectiveness with social and economic healthcare implications (66).

Even modest improvements in risk prediction with a disease as common as CVD translates into thousands of people that may be treated more intensively and could benefit. The progressive rising costs of medical care have increased interest in cost/benefit studies for documenting the economic effects of new tests and therapies. At present, there is insufficient data to document the cost-effectiveness of any of the emerging risk markers beyond traditional lipid tests (67). The residual risk in statin-treated patients has increased interest to the clinical use of apoB as an index of treatment efficacy (68). A large proportion of treated patients achieving their LDL-C and even non-HDL-C targets fail to meet their apoB target without more aggressive therapy (69). In the US adult population over a 10-year treatment period, it was estimated that a non-HDL-C targeting strategy would prevent approximately 300,000 more CVD events than a LDL-C strategy, whereas an apoB strategy would prevent approximately 500,000 more events than a non-HDL-C strategy (70). With the availability of less-expensive generic statins, the cost-effectiveness of intensifying pharmacological intervention aiming to reduce apoB has been enhanced. The measurement of a single marker (apoB) is at least equally cost-efficient for follow-up of patients as the traditional lipid profile with four markers (TC, TG, HDL-C, LDL-C) (71). Thus, although the lipid profile will remain essential for the initial diagnosis and risk categorization, follow-up of dyslipemic patients could be simplified and expenses reduced if only apoB were measured (71). In most countries it is unrealistic to measure apolipoproteins because of lack of reimbursement; simple calculation of non-HDL-C is a low-cost and feasible alternative to apoB in these countries (72).

Interlaboratory and interprofessional collaborations

The production of guidelines on its own is futile; it should always be accompanied by an implementation plan. This includes, at the national level, the mobilization of all parties involved in CVD prevention. Laboratory professionals should identify opportunities for cooperative multidisciplinary approaches to the management of dyslipidemia (Table 3). This can be done by collaborating with different healthcare professionals in the implementation of practice guidelines, and by organizing multidisciplinary seminars or clinical conferences with interactive participation of laboratory professionals and clinicians. Educational initiatives should be part of the implementation plan.

Translational research partnerships are crucial for the introduction of novel promising biomarkers in the clinical laboratories (66); one example is the Asklepios Study, a longitudinal population study designed to investigate the clinical validity and clinical usefulness of biomarkers of preclinical atherosclerosis (73).

The use of epidemiologically established cutpoints for clinical decision-making raises expectations for the analytical performance, underscoring the need for standardization of assays and harmonization of patient results. This requires continuous oversight and accuracy-based surveys of assay reagents, calibrators, and instrumentation changes over time that can affect the quality of testing (74).

The IFCC, EFCC, and related regional federations should take responsibility for the laboratory aspects of the guidelines. This task can be accomplished by joining interprofessional cooperations of cardiologists and other clinical specialties involved in guideline development. This multidisciplinary team-work model allows continuous and bidirectional exchange of knowledge and information. Laboratory professionals could add incremental value to guideline development through their expertise in analytical aspects, such as analytical quality and standardization of the tests, biovariability, interferences, pre-analytical issues, and diagnostic performance characteristics of the tests (sensitivity, specificity, predictive values). Working groups can serve as an advisory board to in vitro diagnostic manufacturers in the strategic choice and development of (novel) CVD biomarker assays. Through communications to the national Clinical Chemistry societies, and organizing conferences, the IFCC and its regional federations could take responsibility for the worldwide implementation of the appropriate guidelines in laboratories. This should result in harmonization of lipid profile reports, measurement units, updated risk cutpoints, and therapeutic targets across different countries within the geographic areas to which the guidelines can be applied.

Conclusions

The post-analytical phase of lipid testing provides important opportunities for delivering clinical added value services in cardiovascular prevention. Without comprehensive laboratory testing of lipoprotein-related risk, high-risk patients can be missed by just measuring TC and LDL-C. Reliable risk assessments can be made only after the essential risk factors, the analytical quality, and lipid profiles have been harmonized and the risk scores have been generally accepted. The appropriate government agencies, professional scientific
societies, in vitro diagnostic manufacturers, and laboratory professionals must work together to ensure the success of implementing the guidelines. Novel biomarkers, such as apoB and non-HDL-C appear to add significant diagnostic and prognostic information to traditional lipid testing in epidemiologic studies and interventional trials, which might reduce (post-)post-analytical errors by integrating these markers in CVD prevention strategies. Large-scale outcome studies and cost/benefit studies are needed to establish the added benefit of these novel biomarkers over standard lipid assessment for screening persons at risk for CVD or monitoring responses to lipid-lowering therapies. In particular, we need to confirm if this additional information helps the healthcare provider to identify additional persons who will develop CVD despite normal or low LDL-C, or to reduce residual risk in statin-treated patients who have achieved their LDL goal.

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Dr. Michel Langlois, born October 13, 1967 studied Medicine at the University of Ghent, Belgium and specialized in Laboratory Medicine with emphasis on clinical chemistry and cardiovascular diagnostics. He presented his PhD thesis on the haptoglobin polymorphism in 1997, followed by his postdoctoral research on iron metabolism and oxidative stress in 1998–2001. Since 2001, he continued his activities in clinical chemistry and lipidology at AZ St-Jan hospital, Bruges, combined with a position of Professor at Ghent University (since 2009). Prof. Langlois is current President of the Royal Belgian Society of Clinical Chemistry (RBSCC), Vice-President of the Belgian Lipid Club (Belgian Atherosclerosis Society), and member of the European Federation of Clinical Chemistry and Laboratory Medicine (EFCC) Working Groups “Cardiac Markers” and “Guidelines”. For his research in risk biomarkers of atherosclerosis he received 9 scientific awards. Prof. Langlois is Editorial Board member of the journal Clinica Chimica Acta and a scientific reviewer for many other international journals including CCLM.