LIPOPROTEINS AND VASCULAR DISEASES DIVISION NEWS
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AACC Better health through laboratory medicine.

INSIDE THIS ISSUE:

CHAIR'S CORNER

Welcome to the Spring issue of the LVDD newsletter. I hope all of you have had a pleasant entry into 2016. With the start of the New Year the configuration of the executive team of LVDD also changed. I am happy to announce our new Treasurer, Mohmed E. Ashmaig, PhD, and Secretary Rojeet Shrestha, PhD. Dr. Ashmaig currently works at Prism HealthDx Inc as senior scientist leading research & development and laboratory operations. He has over 20 years of experience in clinical laboratory science. Dr. Shrestha is a Post-Doctoral Fellow at Hokkaido University in Sapporo, Japan. Finally, Amy Saenger PhD, our past Secretary for LVDD was elected as the chair-elect. Dr. Saenger is an Associate Professor at Department of Laboratory Medicine and Pathology at the University of Minnesota. Please join me in welcoming and congratulating all new and old members of the LVDD executive group.

We have an exciting and very interesting year ahead of us. Again this year we will be hosting you all during the upcoming Annual AACC meeting in Philadelphia with two very interesting symposia. Our main event is the Monday night dinner meeting titled "Current Topics in Cardiovascular Diseases". The key note speaker at this event will be Professor Daniel Rader MD, Chief of Translational Medicine and Human Genetics from University of Pennsylvania. Dr. Rader will update the audience on recent advances in the lipoprotein metabolism field. This symposium will also present the Cooper award to a recipient for outstanding contributions to services in the area of lipoproteins and vascular disease. Finally, top three abstracts submitted to the lipoprotein metabolism field to AACC will be awarded after oral presentations.

The Tuesday dinner symposia, International Lipoprotein Standardization Forum, will address standardization requirements for methods used for testing lipoprotein particle numbers, such as NMR, electrospray differential mobility analysis, isotope dilution MS, and immuno-turbidimetry. The key note speaker for this event will be Vincent Delatour, PhD. who heads up the Biomarkers Research group at the National Metrology Institute in France. He will present the results of the BioSITrace cross-platform comparison study. We hope to follow his presentation with a panel discussion with representatives from companies involved in this study.

Finally, I am excited to announce the results of the year-long efforts by AACC to examine its governance framework. The AACC Board of Directors approved a new governance framework in November 2015 creating a new structure featuring a Science & Practice Core Committee comprising of the chairs of all AACC Divisions. This new committee will serve as the focus for AACC activities in research, science and the translation of science into practice. The AACC leadership has come to the conclusion that strong Divisions are essential to AACC's future. Hopefully, this will provide a seamless interface with other AACC leaders and an opportunity for networking through collaborative activities. I will keep you all updated on the progress and activities taking place in this new governance system.
I hope you all have a wonderful spring and I look forward to meeting you all in person this summer at the upcoming AACC meeting in Philadelphia.

Best wishes,
Amar A. Sethi, MD. PhD.

EDITORIAL

The common misconception that components of a routine lipid panel (LDL-C, HDL-C, and triglycerides) are accurate and well-standardized persists. Miller and colleagues clearly demonstrated inadequate precision and accuracy with direct LDL-C and HDL-C assays [Clin Chem 2010; 56:977-986] with total error ranging from -13.5% to 31.9% among direct LDL-C assays and -19.8% to 36.3% among direct HDL-C assays. This landmark study also demonstrated the major challenge in standardizing LDL-C and HDL-C assays—these lipoproteins are a class of particles that vary in composition and size. They are not, like total cholesterol, characterized by a defined, chemical structure. Biochemically measuring a specific component (cholesterol) from an ambiguous molecular structure like a lipoprotein is inherently difficult if not impossible.

In this issue of the LVDD Newsletter, Drs. Berna Aslan and Paul Yip, from the Institute for Quality Management in Healthcare in Toronto, and the Department of Laboratory Medicine and Pathobiology, University of Toronto and University Health Network, discuss the results of a Canadian accuracy-based lipid proficiency testing program. Aside from the very important discussion about which performance criteria are appropriate, these data continue to show that, while total cholesterol measurement remains robust and triglyceride measurement may be adequate, direct HDL cholesterol assay remain problematic. And if HDL cholesterol measurement is not reliable, accuracy of the Friedewald equation is suspect.

Total cholesterol measurement is relatively well standardized and precise; however, there are important limitations in measurement of both HDL-C and triglycerides. It is important for all of us to recognize the limitations of lipid and lipoprotein measurement, and share data such as this with colleagues, and work with manufacturer's to continue to work on quality improvement and standardization.

Last, we are also excited to welcome newly elected members to the LVDD Executive Committee. Dr. Amy Saenger, Dr. Rojeet Shrestha, and Dr. Mohamed Ashmaig have been elected as Chair-Elect, Secretary, and Treasurer, respectively, and we welcome these talented and respected new members to the leadership team. Welcome also to Dr. Amar Sethi who takes over as Chair. We look forward to a productive year with the new leadership.

John Contois and Mahesheema Ali

Accuracy-based Lipid Proficiency Testing Programs using Performance Criteria Based on Biological Variation: A Canadian Perspective

[1]Berna Aslan and [2]Paul M. Yip

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After the publication of the report "To Err is Human" by the Institute of Medicine of the National Academies, preventing medical errors became the focus of several levels of healthcare quality improvement initiatives. It is estimated that 70% of medical decisions are made based on laboratory test results[1], and the possibility of laboratory-related diagnostic error can lead to decreased patient satisfaction and increased healthcare costs.

To reduce laboratory errors, several programs have been in place for many years, both in the US and Canada, including programs for laboratory accreditation and proficiency testing. Accreditation ensures that laboratories have processes and procedures in place for the management and technical aspects of the work, while proficiency testing programs monitor and provide feedback on the performance of the outcome of these activities.

Proficiency testing (PT) programs, also known as external quality assessment (EQA), can be defined as the formal evaluation of the performance of laboratory tests in terms of inter-laboratory comparisons.[2] Basically, a PT survey consists of sending a set of simulated clinical samples from a PT provider to participant laboratories to test for pre-determined analytes.[3] Next, the PT provider collates results from the laboratories and assesses their performance. The usual approach uses an assigned (target) value and an allowable dispersion around the assigned value to evaluate the participants' analytical performance.

PT providers use different approaches in the determination of assigned values and the allowable limits for acceptability. The mean of participants' results or reference laboratory results can be used as the assigned value. Allowable dispersion around the assigned value (also known as analytical performance specifications, total allowable error, allowable performance limits) may be defined by regulations (e.g. Clinical Laboratory Improvement Amendments [CLIA]), recognized bodies (e.g. NGSP), multiples of the standard deviation based on participants' results, or other pre-determined limits conforming to fit-for-purpose specifications.

While the CLIA total allowable error for PT grading could be considered as an example of the pre-determined fit-for-purpose criteria, it has remained mostly static. There is room for improvement given the non-uniform standards in the fit-for-purpose criteria that have been developed by different organizations. These include one or a combination of the following strategies that follow the Stockholm consensus statement: use of the test in the specific clinical settings, effects on clinical decision-making (biological variation, clinician opinion), recommendations from professional expert groups, regulatory bodies and current state of the art as demonstrated by PT/EQA program providers.[4,5,6]

Assessment of analytical quality based on clinical outcomes in specific clinical situations is the best strategy that establishes the connection between analytical performance and effective patient care. There are, however, only a few studies for a limited number of analytes.[5] In the absence of specifications based on rigorous studies, biologically-based performance goals for imprecision, bias, and total allowable error can provide objective benchmarks for PT grading but also clinical needs.[7] Analytical quality specifications based on biological variation are readily available for a wide range of analytes, although perhaps under-appreciated.

In the case of lipid testing, there is a correlation between high serum cholesterol levels and increased risk of coronary artery disease. Effective diagnosis and treatment of dyslipidemia relies on its measurement. As a result, serum cholesterol has become a focus for laboratorians for more than 50 years to improve the assay variability observed for cholesterol, as well as for triglycerides, HDL- and LDL-cholesterol measurements. This triggered the implementation of the Lipid Standardization Program that aims to develop reproducible and accurate measurement methods and reference materials.[8] PT programs have always been used as a method to monitor the effectiveness of these standardization efforts, and more recently accuracy-based surveys have pushed assessment further to strive towards optimal performance goals.

Accuracy-based proficiency testing programs utilize commutable samples and reference laboratory results to assess the accuracy and precision of routine clinical methods. The Institute for Quality Management in Healthcare (IQMH) in Toronto, provides ISO 17043:2010 accredited PT programs for lipids within Canada and internationally. As an audit of assay performance, we retrospectively reviewed the results of this PT program in an effort to compare the analytical performance of the state-of-the-art of routine clinical laboratory methods with the desirable and optimal performance goals based on biological variation.[9] LDL-C was omitted as most participant laboratories report calculated LDL-C.

Twenty-nine PT surveys distributed between June 2005 and December 2014 were included. PT samples consisted of single donor sera from healthy individuals with no additives from Interstate Blood Bank (Memphis, Tennessee). Each unit of blood is collected into sterile plain bags. After collection, red cells are separated and removed from the serum, and then serum is held at room temperature for a minimum of 48 hours to allow coagulation. Serum is re-centrifuged to remove any residual visible material and stored and shipped at 1°C to 10°C to Dynacare Laboratories (Ontario, Canada) where the serum is aliquoted and shipped to participants. Homogeneity and stability testing is performed using the algorithm recommended in ISO 13528:2005.[11] Although this process for the preparation of commutable materials is not as rigorous as described in CLSI document C37-A[12], it is sufficient to provide minimally processed specimens to enable accuracy-based grading.

Two challenges are distributed in each survey and the surveys are shipped three times per year. Participants' results were assessed against the values obtained at Northwest Lipid Metabolism and Diabetes Research Laboratories (Seattle, Washington), which uses CDC certified methods. Robust statistics based on ISO 13528:2005 were used to calculate peer group means and standard deviations to eliminate outliers' effects.

Cholesterol: Cholesterol was assessed across all PT samples with 374 peer groups across seven major manufacturer platforms. Average method bias was –0.23% (range: 19.4%–6.5%), and average CV was 1.9% (range: 0.4%–11.3%). Cholesterol concentrations of the survey materials ranged between 117–365 mg/dL (3.0–9.4 mmol/L). Ninety-five per cent of the peer group biases and 96% of the CVs were within the biological variation based desirable performance targets of 4.1% and 3.0%, respectively. Certainly, the routine methods have improved over time and now are able to meet the desirable goals originally set forth by the NCEP and conform to the CLIA requirements for the most part. The all-methods' mean (AMM) showed a very good correlation with reference results, which further demonstrates that standardization has been effective for cholesterol testing.

Triglycerides: Triglycerides were assessed in 362 peer groups across the same major platforms. Average method bias was –3.3% (range: –23.8%–12.2%), and average CV was 2.8% (range: 0.4%–14.8%). Ninety-five per cent of the peer group biases and 99% of CVs were within the desirable limits of 9.6% and 10%, respectively. The overall imprecision of routine methods appears superior to the performance specification based on biological variation. Then again, the bar set by the NCEP guidelines was already high, approaching the optimal goals for both imprecision and accuracy. AMMs (range 45-1616 mg/dL; 0,51-18,25 mmol/L) were correlated very well with reference lab results and differences between all method means and reference results varied between –48–80 mg/dL (–0.54–0.90 mmol/L). The high variation in bias is tolerated since the CVs generally remained tight except as values decreased <100 mg/dL

HDL-Cholesterol: HDL-C was evaluated in 372 peer groups across the same major platforms. Average method bias was 0.7% (range: –34.6%–105.4 %), and average CV was 4.0% (range 0.7%–40.2%). Sixty-nine per cent of the peer group biases and 66% of the peer group CVs were within the biological variation based desirable performance limits of 5.6% and 3.7%, respectively. Of the three commonly measured lipids, HDL-C had the poorest and most varied performance. This should not be surprising given the quality gap in the CLIA requirement of ±30% and the desirable goal of 11.6% based on biological variation. Even with a tighter NCEP guideline specification, there is no incentive to improve so long as laboratories can pass their PT challenges.

Conclusion: Overall biases and CVs for cholesterol and triglycerides were within the desirable limits. The performance of the majority of clinical testing meets a fit-for-purpose specification that is more objectively based on biological variation. Cholesterol testing is positioned to improve further if assays can achieve the optimal performance goals thus making it six sigma quality relative to CLIA. However, with HDL-C assays, there were peer group bias and CV values exceeding these limits indicating poor method standardization and

reproducibility. Statistically speaking, one could repeatedly measure HDL-

C enough times to get a reliable value, but it would be unrealistic to have a patient return for blood draws before deciding on a treatment. The IQMH PT program for lipids sets the HDL-C allowable performance limit at ±10% of the reference value. Although this may be criticized as being rather tight, it is grounded in a specification based on biological variation. With the widespread testing of lipids in the treatment and prevention of cardiovascular diseases, continued efforts to improve the analytical performance of lipid tests is needed to support better quality for patient care.

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Table 1. Desirable and optimal analytical performance goals for lipid tests based on biological variation.[9]

Analyte	BV Imprecision Goals (CV%)		BV Accuracy Goals (%)		BV Desirable Total Error (%)
	Desirable	Optimal	Desirable	Optimal	
Total Cholesterol	3.0	1.5	4.1	2.1	9.0
Triglycerides	10.0	5.0	9.6	4.8	26.0
HDL-C	3.7	1.8	5.6	2.8	11.6

Table 2. Comparison of CLIA proficiency testing criteria for acceptable analytical performance and NCEP desirable performance guidelines for lipid tests.[10]

Analyte	IQMH Total Allowable Error*	CLIA Total Allowable Error	National Cholesterol Education Program Guidelines		
			Precision Goal (CV)	Accuracy Goal	Total Error
Total Cholesterol	±5%	±10%	≤ 3%	≤ 3%	≤ 9%
Triglycerides	±10%	±25%	≤ 5%	≤ 5%	≤ 15%
HDL-C	±10%	±30%	≤ 4%	≤ 5%	≤ 13%

* At the time of collection of data presented in this article

JOURNAL WATCH

Mora S, Caulfield MP, Wohlgemuth J, et al. Atherogenic lipoprotein subfractions determined by ion mobility and first cardiovascular events after random allocation to high-intensity statin or placebo: Circulation 2015; 132:2220-2229.

Ion mobility (IM) is a novel technique for direct measurement of lipoprotein particle subclasses. In the present study, IM was used to measure lipoproteins in 11,186 participants of the JUPITER study, a randomized controlled study of men ≥50 years and women ≥60 years with LDL cholesterol <130 mg/dL and hsCRP ≥2.0 mg/L. Subjects were randomized to rosuvastatin 20 mg/day or placebo. After a maximum follow up of 5 years there were 307 first cardiovascular events and 522 deaths. In the placebo group apolipoprotein B [HR 1.28 (1.11-1.48)], nonHDL cholesterol [HR 1.18 (1.01-1.38)], IM-measured nonHDL particles [HR 1.19 (1.05-1.35)], and IM-measured LDL particles (HR 1.21 (1.07-1.37)] predicted cardiovascular events. On treatment, LDL cholesterol, nonHDL cholesterol, apoB, HDL cholesterol, apo AI, and IM-measured VLDL subclasses, LDL particles, IDL particles and large LDL particles all predicted CVD events and all-cause deaths.

Wassef H, Bissonnette S, Saint-Pierre N, et al. The apoB-to-PCSK9ratio: A new index for metabolic risk in humans. J Clin Lipidol 2015; 9:664-675.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) loss of function is known to protect against cardiovascular disease. These data suggest that lower levels of PCSK9 may also increase apoB lipoproteins into adipose tissue and induce metabolic dysfunction. In this study of normoglycemic men (n=33) and postmenopausal women (n=48), aged 45-74 years, plasma apoB but not LDL-C or PCSK9 concentrations correlated with fasting and postprandial triglycerides, chylomicron clearance, glucose-stimulated insulin secretion, and inversely correlated with insulin sensitivity and white adipose fat lipoprotein lipase activity. Using regression analysis, the apoB-to-PCSK9 ratio was most strongly correlated with these biomarkers of adipose tissue dysfunction and insulin resistance. Although we cannot infer causation from this association study, these data raise important questions about the role of PCSK9 inhibition and risk of diabetes. The effect of PCSK9 loss of function or inhibition clearly lowers LDL particle concentrations, largely via liver clearance, but adverse effects via nonhepatic clearance of apoB particles remains an important area for research.

Miller PE, Martin, SS, Toth PP, et al. Screening and advanced lipid phenotyping in familial hypercholesterolemia: The Very Large Database of Lipids Study-17 (VLDL-17). J Clin Lipidol 2015; 9:676-683.

The authors report data culled from >1.3million subjects who underwent testing with the Vertical Auto Profile (VAP) panel from Atherotech. Subjects with presumptive familial hypercholesterolemia (FH) were identified using LDL cholesterol cut points recommended by the National Lipid Association and percentile-equivalent cut points for "biologic" LDL cholesterol [LDL fraction without IDL cholesterol and Lp(a)-cholesterol]. LDL-cholesterol was calculated using the Friedewald equation; biologic LDL-cholesterol was directly measured from VAP. Using the Friedewald equation, 3829 patients met the NLA criteria for FH and, of these, 79% were at or above the equivalent cut point for biologic LDL cholesterol. Interestingly, 63% and 37% of screen positive patients also had elevated IDL cholesterol and Lp(a)-cholesterol, respectively. The limitations of this study include (1) unknown fasting status of the patients and (2) lack of true FH diagnosis for these subjects, but these data suggest that the Friedewald equation is robust in screening potential FH patients.

Lee CH, Woo YC, Lam JKY, et al. Validation of the Pooled Cohort equations in a long-term cohort study of Hong Kong Chinese. J Clin Lipidol 2015; 9:640-646.

The American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommends a global cardiovascular disease risk score to help classify patient risk and help with therapeutic decisions. The new Pooled Cohort equation was developed with data from five large epidemiological studies with men and women from multiple racial groups. Despite the racial/ethnic diversity of the database, questions remain about the suitability of the ACC/AHA risk equation for different racial and ethnic groups. In this study the ACC/AHA

Pooled Cohort equation was tested in 2895 Chinese men and women, aged 25-74 years, from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) and compared to the Framingham cardiovascular risk equation. After a median follow up of 10 years 122-138 subjects developed cardiovascular disease, depending on the model. The calibration scores for both the Pooled Cohort and Framingham risk equations were inadequate in men but acceptable in women. Risk in men was significantly overestimated by both models while risk was underestimated in women with the Pooled Cohort equation. Recalibration of the model with CRISPS data improved the performance of the models. These data illustrate the difficulty in finding a universal risk prediction model, but do not negate the validity and clinical utility of the Pooled Cohort equation for use by multiple ethnic groups in the US.

Gidding SS, Champagne MA, de Ferranti SD, et al. The agenda for familial hypercholesterolemia: A scientific statement from the American Heart Association. Circulation 2015; 132:2167-2192.

This excellent review highlights the challenges of identifying and treating familial hypercholesterolemia (FH). From a public health standpoint FH remains largely underdiagnosed and undertreated. Individual patients with FH are often treated for elevated cholesterol without appreciation of the familial and genetic implications of the disease. This scientific statement touches on the genetics and pathobiology of the disease and describes best practices for screening, diagnosis, and treatment.

LIPOPROTEINS AND VASCULAR DISEASES DIVISION NEWS

We hope you enjoy this edition of The LVDD Newsletter. Your feedback is welcome. We also encourage you to submit review articles, original research, opinions, and other information you wish to share with your colleagues.

We are especially interested in a volunteer to prepare annotated bibliographies for the Journal Watch section. A brief review of the most important articles related to lipid and lipoprotein measurement, cardiovascular biomarkers, and outcomes is an important service to our members.

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