Dear LVDD members:

I hope you had a great time at the Annual AACC Meeting held in Atlanta this July. In keeping with our tradition LVD Division had exceptional educational events at this meeting. These events included three invited talks by experts in the field of lipoproteins and atherosclerosis and presentations by the first authors of the 3 best abstracts selected by LVDD executive committee. The Annual LVDD Dinner Meeting, where current topics in cardiovascular diseases are traditionally presented, was highlighted by excellent and informative talks by Drs. Peter Wilson and Sridevi Devaraj. Dr. Wilson from Emory University, who is a renowned cardiovascular epidemiologist and past investigator of the Framingham Heart Study, discussed a fascinating topic: Cholesterol – Past, Present, and Future. This talk was followed by the Zak Award presentation. Dr. Sridevi Devaraj from Texas Children’s Hospital and Health Centers was felicitated with this prestigious award for her outstanding contribution to research in lipoproteins and vascular diseases. Dr. Devaraj gave a very informative talk entitled: Non-traditional biomarkers of CVD subsequent to the acceptance of the award. The International Lipoprotein Standardization Meeting held on July 28 was also well attended. At this meeting Dr. Christopher Naugler, Associate Professor, University of Calgary, Canada presented an important lipid testing related topic entitled “Fasting time and lipid levels in a community-based population: A cross-sectional study”. This was followed by 3 poster presentations. This program was held in collaboration with Informatics Division. The two meetings were sponsored by Denka-Seiken, Diazyme, Helena, and Randox companies.

It is with great pride I announce that LVDD has resumed its formal quarterly newsletter “LVDD Newsletter” replacing “Fats of Life”. Drs. John Contois and Mahesheema Na have led this effort and the LVDD Executive Committee thanks them for their hard work. The main purpose of the Newsletter is to keep all members of LVDD up to date with the recent research in lipoproteins and vascular diseases. Please consider contributing to this newsletter by writing original articles, review articles, summary of recent literature etc. As you may be aware AACC has opened a web based forum to all its members called “AACC Artery”. You can access this through the following link: https://community.aacc.org. AACC Artery is an online community where AACC members can engage with each other to find answers to questions, share knowledge, and refine ideas as scientific practice and research issues, lab management, and career development. I encourage you to take advantage of this benefit of AACC membership. We are also considering establishing a separate group for the LVD Division.

Dr. Amar Sethi, MD, PhD. will be the new Chair of LVD Division beginning January 01, 2016. The LVDD is scheduled to elect the following positions by December 1, 2015: Chair-Elect, Secretary, and Treasurer. If you are interested or if you know any other LVDD members are interested in these positions please nominate them by contacting our Continued on page 2
EDITORIAL

“FATS OF LIFE”

Welcome to the first edition of the LVDD Newsletter. Although the title, Fats of Life, is gone we hope the spirit of “Fats” continues with this new volume. For those of us who are longtime LVDD members, Fats of Life was a valued perk of membership. It provided so much more than brief snippets of division news typical of most newsletters. “Fats” allowed academic members to share their research, industry members to describe new and innovative products, and it showcased division activities and allowed leadership to recognize member’s achievements and awards. The quality of many of the articles belied the fact that this was a simple newsletter; it was in fact a great source of information. To many of us the newsletter was the glue that held the division together. Mahesheema and I hope to continue the tradition and quality of the Fats of Life with our new division newsletter.

I believe the newsletter was given its name, “Fats of Life”, by its first Editor, Don Wiebe, who developed and molded the newsletter in its infancy. Russ Warnick followed as Editor and really shaped the newsletter and made it successful. I was fortunate to serve as Associate Editor with Russ for many years and contribute to the success of the newsletter. Many members shared with me their pleasure in receiving the newsletter every quarter, and I was very proud when ‘Fats’ was recognized with the AACC education award. The articles that appeared in our newsletter were equal to the quality of manuscripts published in many peer reviewed journals.

Like most things in life, success is often due to the efforts of one person. Russ championed the newsletter and drove its success. When Russ stepped down as Editor he left a void. Mahesheema and I have accepted the challenge to continue the tradition of a strong division newsletter, and we have identified topics and authors that will once again make the newsletter a “must read” for our members.

In this, our first issue, we have an outstanding article from Dr. Matt Kelso and colleagues from Medpace Reference Laboratories describing accuracy of LDL cholesterol measurement at concentrations less than 70 mg/dL—an issue in the era of aggressive LDL lowering.

We will continue to strive to find additional high quality articles and we encourage all of you to submit your research, opinions and reviews to us to share with your colleagues in the division. We also want to hear your opinion about our new newsletter. Please share your comments with either Mahesheema or me.

John Contois and Mahesheema Na
Editors, The LVDD Newsletter

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Secretary Dr. Ping Wang at Pwang@houstonmethodist.org.

Finally, I request you all to renew your LVDD membership for 2016 to show your continued support. If you have discontinued your membership please consider to become member again. The benefits are enormous!!!

Best wishes,

Kris Kulkarni, Ph.D.
Accurate Assessment of LDL Cholesterol Reduction at Levels below 70 mg/dL has Implications in the Estimation of Efficacy for New Drugs in Development

Matthew L Kelso PhD,1, Christopher Daniels PhD1, Nan Plunkett BS1, Rong Zhou PhD2, Miriam Zangmeister MS2, Christine Fritz MS1, Traci Turner MD*1, Evan Stein MD, PhD†1. Medpace Reference Laboratories, 5365 Medpace Way, Cincinnati, Ohio, 45227. US 1Medpace Biostatistics, Cincinnati, Ohio US

Introduction

PUC is considered the “gold standard” method for LDL-C measurement (LDL-Cp),1 but is not readily available in many laboratories because it is a labor-intensive protocol requiring specialized equipment. Friedewald et al2 reported a cost-effective method for calculating LDL-C (LDL-CF) which has gained widespread acceptance when TG are < 400 mg/dL. While the Friedewald formula was originally validated in patients with LDL-C >70 mg/dL and has proven robust and reliable above this level, its accuracy and validity for lower LDL-C levels has recently been questioned.3-4 This may have significant implications for both combination lipid modifying therapies currently available and those in development. Current therapies have demonstrated cardiovascular risk reduction directly attributable to lowering LDL-C below those levels5 whereas LDL-C lowering compounds in development, such as the recently approved proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, alirocumab and evolocumab, have been shown to achieve very low levels.6-7 An alternative formula, the “Hopkins” formula (LDL-Ch), has been proposed to address the shortcomings of the Friedewald formula. The Hopkins formula uses a variable TG:VLDL-C ratio (varying from 3.1 to 11.9) dependent on total cholesterol (TC), TG, and non–HDL-C levels5 rather than the fixed ratio of TG/5 used by Friedewald. However, Hopkins has not been validated against PUC. As an alternative to the calculated methods, homogenous methods (LDL-Cc) are detergent based assays and were originally introduced to measure LDL-C where TG >400 mg/dL or patients were non-fasting. However the performance of these assays vary by manufacturer and from reagent generation within the same manufacturer. Additionally, accuracy relative to PUC has also been shown to deteriorate in diseased (primarily dyslipidemic and cardiovascular) populations and there is no data on accuracy at low LDL-C concentrations.9

Here, we report the results of LDL-C measured by PUC where: LDL-C = TC – (HDL-C + TG/ adjusta-

Methodology10

Samples

Serum or plasma samples were collected after an overnight fast (water only) and analyzed for TC, TG, high density lipoprotein cholesterol (HDL-C), LDL-Cp and LDL-CF and LDL-Ch and were evaluated for those with TG ≤400 mg/dL, resulting in total of 1299 comparisons. The samples were from patients in a specialized lipid clinic or participants in clinical trials, and included pediatric patient samples. All samples were received de-identified of demographic information.

Analytical methods

TC, the cholesterol content of isolated fractions, and TG were measured at Medpace Reference Laboratories, Cincinnati, OH, which maintained CDC-NHLBI Lipid Standardization Program Part III throughout the entire testing period (Participant number LSP-395).11 Analysis of TC and TG was by enzymatic methods on a Beckman Coulter AU Series automated chemistry analyzer with in-house developed serum calibrators directly traceable to CDC-NHLBI reference procedures.11 LDL-Cp was performed using the method modified from the Lipid Research Clinics methods manual.12 Briefly, serum or plasma was overlaid with normal saline (density 1.006 g/mL) and centrifuged (Beckman Ultracentrifuge Model # L-90K and rotor, Type 50.4) at 40,000 rpm for 18–22 hours at 10°C to separate VLDL-C in the supernatant (top fraction) from LDL and HDL in the infranatant (bottom fraction). The cholesterol concentration of the infranatant was measured. All apolipoprotein B-containing lipoproteins, VLDL-C, intermediate density lipoprotein (IDL), LDL, and Lp(a), were precipitated from serum using 50 kDa dextran sulfate with magnesium ions (MgCl2),13 and the cholesterol in the remaining HDL fraction was measured. The HDL-C concentration was subtracted from the infranatant cholesterol to provide the LDL-Cp value. VLDL-C was calculated by subtracting the “bottom” fraction cholesterol from TC. The ratio of cholesterol in VLDL to TG was calculated by VLDL-C/TG.

Calculated LDL-C was estimated from the Friedewald formula2 where: LDL-Cp = TC – (HDL-C + TG/5) and from the Hopkins formula where: LDL-Ch = TC – (HDL-C + TG/ adjustable factor mg/dL); the adjustable factor was determined as the strata-specific median TG:VLDL-C ratio.8 LDL-Cc was measured by a homogeneous enzymatic assay using Roche C.f.a.s. Lipid Calibrator and LDL-C plus 2nd generation reagent (both traceable to the Cholesterol Reference Method Laboratory Network accuracy base for LDL-C) on a Beckman Coulter AU Series automated chemistry analyzer.

Statistical methods

Summary statistics, mean ± standard deviation (SD) values for continuous variables, and numbers of patients and percentages for categorical variables were calculated on measured and calculated lipid parameters. Subgroup analyses based on the differences between LDL-Cp, LDL-Ch, LDL-Cc, and LDL-C as compared to LDL-Cp for each sample were performed based on LDL-Cp and TG levels at selected cut-points.
Similar analysis was done for VLDL-C/TG ratio. The percent difference for each of the measurement methods from PUC at LDL-C ≤ 100 mg/dL are presented in difference plots.

Results

Overall results for the 1,299 samples are shown in Table 1. LDL-Cr ranged from 2 - 453 mg/dL. The ranges for the other measurement methods were similar; 0 - 449 mg/dL by Friedewald, 1 - 446 mg/dL by Hopkins, and 7 - 369 mg/dL by the direct method. This corresponded to an overall difference (mean ± SD) of -18.9 ± 19.34%, -9.3 ± 17.83%, and -0.8 ± 21.91% for Friedewald, Hopkins, and the direct method, respectively. TG ranged from 28 to 394 mg/dL. Assessment based on selected PUC LDL-C cut-points (Table 2) resulted in 947 results ≤70 mg/dL, 860 results ≤50 mg/dL and 322 results ≤25 mg/dL.

The Friedewald formula underestimated LDL-C as compared to LDL-Cr at all LDL-C cut-points (Table 3). LDL-Cr showed a minimal difference of -3.4% when LDL-C was between 101-200 mg/dL. As values decreased below 100 mg/dL, the difference between Friedewald and PUC progressively increased to 6.9% between 100 and 71 mg/dL, 14.3% between 70 and 51 mg/dL, 20.9% between 50 and 26 mg/dL, and 32.9% at 25 mg/dL or below (Figure 1). Within each LDL-C cut-point the difference between Friedewald and PUC increases for every 100 mg/dL rise in TG, especially at LDL-C below 50 and 25 mg/dL (Figure 2).

Overall, the Hopkins method underestimated LDL-C as compared to PUC at all LDL-C cut points (Table 4), though to a lesser degree than as estimated by Friedewald. The underestimation using LDL-Cr increased as LDL-C levels decreased; 2.2% between 100 and 71 mg/dL, 2.3% between 70 and 51 mg/dL, 9.3% between 50 and 26 mg/dL and 19.7% at 25 mg/dL or below (Figure 3). For TG levels ≤200 mg/dL, Hopkins underestimated LDL-C at all LDL-C cut-points (overall mean difference 15.5% for TG ≤100 mg/dL, 8.2% for TG 101 to 200 mg/dL) and overestimated LDL-C when TG levels were ≥201 mg/dL (overall mean difference 6.6% for TG 201 to 300 mg/dL, 20.3% for TG 301 to 400 mg/dL), shown in Figure 4.

As compared to PUC, LDL-C measured with the “direct” method was accurate (Table 5) overall with a % difference of −0.8 (p = 0.17). However, the differences at all LDL-C cut-points were statistically significant with underestimation of LDL-C as compared to PUC: 3.7% between 101 and 200 mg/dL, 2.7% between 100 and 71 mg/dL, 4.1% between 70 and 51 mg/dL, and 4.3% between 50 and 26 mg/dL (Figure 5). When LDL-C was ≤25 mg/dL, the direct method overestimated LDL-C by 8.8%. The direct method was more consistent across increasing TG levels (Figure 6).

Conclusions

Compared to PUC, both calculated LDL-C methods and direct measurement methods underestimated LDL-C at prespecified cut-points. While the difference in LDL-C, as determined by the direct method, remained relatively constant across LDL-C cut-points, the calculated methods produced estimates that were progressively low as LDL-C decreased below 100 mg/dL. As determined by the Friedewald formula, increasing TG levels result in increasing bias when LDL-C ≤100 mg/dL, reaching bias levels as high as 65% when LDL-C ≤ 25 mg/dL and TG > 200mg/dL. At TG levels ≤ 200 mg/dL, the Hopkins formula also underestimates LDL-C, though not to the extent of Friedewald. However, Hopkins overestimates LDL-C when triglycerides are >200 mg/dL. Overall, the “direct” homogenous method for measuring LDL-C was more reliable and did not show increasing differences with various TG cut-points. However, this finding cannot be applied to other direct measurement methods as their performance has been reported to vary. For drugs in development, accurate measurement of key efficacy parameters, such as LDL-C, is of paramount importance to assess response to drug. Underestimation of LDL-C may lead to overestimation of treatment effect. Robust clinical trial design is essential for regulatory approval. Recent work demonstrating additional clinical benefit with improved cardiovascular outcomes when LDL-C levels are reduced below previous targets with combination lipid modifying therapies suggests that clinicians should exercise caution when interpreting calculated laboratory values of LDL-C, as under or overestimation of LDL-C levels can lead to erroneous treatment decisions.

References


7. Sabatine MA et al. Efficacy and Safety of Evolocumab in


<table>
<thead>
<tr>
<th>Table 1: Summary Statistics for Lipid Parameters</th>
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<tbody>
<tr>
<td>Lipid Parameter (units)</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
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<tr>
<td>HDL-C (mg/dL)</td>
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<td>TG (mg/dL)</td>
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<td>Calculated LDL-C&lt; by Friedewald (mg/dL)</td>
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<td>Calculated LDL-C&lt; by Hopkins (mg/dL)</td>
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<td>LDL-C&lt; by preparative ultracentrifugation (mg/dL)</td>
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<td>“Direct” LDL-C&lt; (mg/dL)</td>
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<td>% Difference Friedewald&lt;</td>
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<tr>
<td>% Difference Hopkins&lt;</td>
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<tr>
<td>% Difference “Direct”&lt;</td>
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<tr>
<td>VLDL-C (mg/dL)</td>
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Dr. Wilson’s presentation

Presentation of the Zak Award to Dr. Devaraj
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<tr>
<th>LDL-C&lt;sub&gt;p&lt;/sub&gt; (mg/dL)</th>
<th>LDL-C&lt;sub&gt;F&lt;/sub&gt; (mg/dL)</th>
<th>% Difference</th>
<th>LDL-C&lt;sub&gt;F&lt;/sub&gt; (mg/dL)</th>
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<td>&lt;25</td>
<td>18.1 (4.85)</td>
<td>8.8 (37.08)</td>
<td>12.3 (5.67)</td>
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<td>26-50</td>
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<td>28.5 (7.25)</td>
<td>-20.9 (14.69)</td>
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<td>50.9 (9.88)</td>
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<td>71-100</td>
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<td>&lt;.0001</td>
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Note: Overall N=1289 for direct LDL and N=1299 for other parameters.
Figure 3: Difference plot for LDL-C ≤ 100 by Hopkins and Preparative Ultracentrifugation

Figure 4: % Difference (mean +/-SE) in LDL-C (Hopkins) by TG level

Figure 5: Difference plot for LDL-C ≤ 100 by Direct measurement and Preparative Ultracentrifugation

Figure 6: % Difference in LDL-C (Direct) by TG level
JOURNAL WATCH

The NLA Expert Panel released part 2 of its recommendations online. The highlights of Part 2 include:

- Importance of lifestyle changes in cholesterol management, including detailed dietary and physical activity recommendations
- Recognition of the importance of monitoring risk factors in diverse ethnic and racial groups
- Importance of monitoring children and adolescents, especially those with a family history, and
- Recognizing that high risk patients may benefit from additional non-statin therapies such as ezetimibe.


Previous studies looking at the effectiveness of the addition of second drug to statins have shown value in reducing cardiovascular events. This was a randomized, double-blind trial of >18,000 patients hospitalized for an acute coronary event followed for a median of six years. Patients were randomized to simvastatin (40 mg) and ezetimibe (10 mg) (combination therapy) or simvastatin (40 mg) and placebo. LDL-C was lowered to 54 mg/dL or 70 mg/dL with combination or monotherapy, respectively. The event rate (cardiovascular death, nonfatal MI, unstable angina requiring hospitalization, coronary revascularization, or nonfatal stroke) was 34.7% with monotherapy but 32.7% with combination therapy (p=0.016). The addition of ezetimibe to simvastatin resulted in additional cholesterol lowering and improved cardiovascular outcomes.


Previous studies have reported an association of lipoprotein(a) [Lp(a)] and calcific aortic valve disease (CAVD). The authors propose a pathway whereby oxidized phospholipid-rich Lp(a) particles may induce inflammation and calcification of the aortic valve. Apparently, autotaxin from Lp(a) and interstitial cells which is carried by Lp(a) acts as a phospholipase transforming oxidized phospholipids to lysophosphatidic acid, initiating a cascade of events leading to inflammation and calcification.


Although drugs to raise HDL cholesterol have not proven effective in reducing cardiovascular events, data continues to accumulate suggesting that cholesterol efflux capacity (CEC) by HDL particles is an important biomarker. An important prospective study published last year showed that low CEC predicted increased CHD risk (Rohatgi A, et al. NEJM 2014;37:2383). This prospective study of the EPIC-Norfolk cohort also assessed CHD risk associated with CEC in 1745 cases and 1749 controls. CEC was significantly associated with CHD independent of all other demographic and major risk factors, including age, sex, diabetes, hypertension, smoking, BMI, LDL-C, triglycerides, HDL-C, and apo AI [Odds Ratio: 0.64 (0.51-0.80)].


In this trial subjects were infused with different doses of an HDL mimetic to assess kinetics and adverse events. The authors included measurement of cholesterol efflux capacity with the understanding that HDL function is likely more important than changes in HDL cholesterol or apo AI. The hypothesis underlying this study is that direct infusion of lipid-poor apo AI particles will promote interaction with ABCA1 and improve cholesterol efflux capacity. Larger HDL particles, as seen with CETP inhibition, are likely less functional in cholesterol efflux. CSL112 was effective in increasing CEC from macrophages by an average of 3.1-fold at peak compared to baseline, suggesting that use of these lipid-poor HDL mimetics may reduce risk by increasing cholesterol efflux.

“The hypothesis underlying this study is that direct infusion of lipid-poor apo AI particles will promote interaction with ABCA1 and improve cholesterol efflux capacity.”
We hope you enjoy this new 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.

Visit us online at: www.aacc.org/community/divisions/lipoproteins-and-vascular-diseases