LDL Subfractions Analysis in Pro-atherogenic Dyslipidemia

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Abstract

Background
Early recognition of pro-atherogenic risk factors is important for prevention and treatment of coronary artery disease (CAD). The NCEP ATP III guidelines identified LDL cholesterol (LDL-C) as the primary target for CAD therapy and risk assessment. New ACC/AHA guidelines replaced traditional lipid risk factors with a 10-year ASCVD risk calculator weighing heavily on non-lipid risk factors, ignoring a large body of evidence clearly recognizing specific dyslipidemic profiles with increased CAD risk. Numerous studies clearly demonstrate that small dense LDL, VLDL remnants and IDL are independently atherogenic while large buoyant LDL, HDL and possibly large VLDL may not be. Exclusion of such evidence could result in patient misclassification possibly leading to under or overtreatment of individuals. In this study, pro-atherogenic lipoprotein subfractions were measured using the Quantimetrix Lipoprint® LDL system, (Quantimetrix Corporation, Redondo Beach, CA). The test yields critical information for early detection of individuals at risk or with existing CAD and allowing for a more individualized implementation of treatment.

Objective
Demonstrate the benefit of measuring the atherogenic LDL subfractions with the comprehensive analysis on the Lipoprint LDL system and assist clinicians in identifying, stratifying and customizing treatment for those at risk.

Methods
Lipid profiles for a total of 273 recruited subjects were determined by testing their total cholesterol, triglycerides, LDL-C and HDL-C using standard clinical methods. Subjects were segregated into two groups, “normolipidemic” and “dyslipidemic” based on ATP III desirable lipids status. Cholesterol levels in the lipoproteins subfractions, large VLDL, Mid-C (VLDL remnants), Mid-B (large IDL), Mid-A (small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 to LDL-7 (small dense LDL) and HDL were also measured in both groups using the Quantimetrix Lipoprint LDL system, a linear polyacrylamide gel electrophoresis method. Results from the traditional lipid profile were compared to the lipoprotein subfraction profiles obtained by Lipoprint.

Results
The lipid test results, mean and range, for the 273 study subjects were: total cholesterol 196 (104 – 319) mg/dL, triglycerides 96 (25 – 345) mg/dL, LDL-C 117 (58 – 215) mg/dL and HDL-C 55 (26 – 137) mg/dL. Out of the 273 total subjects, 133 (49%) were classified normolipidemic according to the ATP III lipid guidelines while 140 (51%) had at least one parameter outside the recommendations. LDL subfractions analysis by the Lipoprint system revealed that 17 (13%) out of the 133 previously classified “normal” subjects had cholesterol levels outside the 95% confidence interval range for a given LDL subfraction. Of the 141 “dyslipidemic subjects,” 69 (49%) had a normal LDL subfraction distribution. Lower levels of large buoyant LDL-1 were observed in many of the dyslipidemic subjects.

Conclusions
Clinical studies identify small dense LDL, VLDL remnants and IDL subfractions independently associated with increased CAD risk above other lipid factors. Measurement of these highly atherogenic lipoprotein subfractions as demonstrated by the Lipoprint system could be a better predictor of CAD risk than measurement of other traditional lipid risk factors.

Introduction

Dyslipidemias have been recognized as risk factors for coronary artery disease (CAD). The National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) guidelines identified LDL cholesterol (LDL-C) as the primary target for CAD therapy and risk assessment. Although the association between elevated levels of LDL cholesterol and atherosclerosis is universally accepted, cumulative data reveals that a relative high proportion of individuals develop atherosclerosis and heart disease in spite of having LDL cholesterol levels in the normal range while others with elevated LDL cholesterol remain disease free.

After several years of data review from randomized controlled trials (RCTs), the ACC/AHA Task Force released new guidelines base on a 10 year ASCVD risk calculator that weighs heavily on non-lipid risk factors. As a consequence, lipid measurements have become practically inconsequential as CAD risk factors in primary prevention and LCL-C is no longer a target for treatment in secondary prevention. This raises the concerns of possible risk miscalculation that could lead to under-treatment of individuals at risk and overtreatment of individuals at low risk.

It is well established that CAD is a multifactorial disease and hyperlipidemias play an important roll in its development. Clinical correlation studies have demonstrated that the apo B containing lipoproteins, especially LDL-C, are responsible for the atherosclerotic process leading to heart disease. This evidence has lead to the measurement of LDL-C as the main determinant of CAD risk. However, it is well documented that LDL is heterogeneous consisting of multiple subclasses varying in density, particle size, chemical composition, function and atherogenic potential. LDL particles include the highly atherogenic triglyceride enriched VLDL remnants (VLDLr), intermediate density lipoprotein (IDL) and small dense LDL (sdLDL) as well as the non-atherogenic good, large buoyant LDL (lbLDL). This supports the fact that LDL-C may not be a good indicator of CVD risk. The same is true for other CVD risk measurements such as LDL-C, non-HDL-C, apo B, LDL-P, LDL average particle size that include atherogenic and non-atherogenic LDL particles in the same metric. Therefore, measurement of the individual atherogenic and non-atherogenic LDL subclasses could provide more specific assessment of CVD risk than methods that measure all the LDL subfractions combined.

In this study the Quantimetrix Lipoprint LDL system, (Quantimetrix Corporation, Redondo Beach, CA) was used to measure the cholesterol levels in all the lipoprotein fractions and subfractions; that is VLDL-C, Mid-C (VLDLr-C), Mid-B (IDL-C), Mid-A (sIDL-C), LDL-1 and LDL-2 (lbLDL-C), LDL-3 to LDL-7 (sdLDL-C) and HDL-C in 273 randomly selected individuals. Serum samples from the 273 self-declared normal individuals were assayed by the traditional lipid risk factors, that is total cholesterol, triglycerides, LDL-C and HDL-C and the Lipoprint LDL system. Samples were classified normolipidemic or dyslipidemic based on the results obtained by each method. Comparison of the Lipoprint subfraction profiles to the traditional lipid profiles revealed a large degree of discordance and overlapping between normolipidemic and dyslipidemic individuals.

In order to clarify the discordance between the traditional lipid profiles and the Lipoprint subfractions profiles, serum samples from an additional 22 random individuals were obtained and analyzed by the Lipoprint LDL subfractions method and nine other traditional and non-traditional lipid assay methods; that is TC, TG, LDL-C and HDL-C), Non-HDL-C, Total Cholesterol to HDL cholesterol ratio, NMR LDL particle number (LDL-P) and the new ACC/AHA Guidelines ASCVD Risk Calculator.

The findings from this study show significant discordance between the various methods that could result in CVD risk overestimation or underestimation depending of the test method used. The study suggests that measurement of the specific atherogenic and non-atherogenic LDL subfractions may offer a more specific assessment of the individuals at risk for CVD.

Materials and Methods

Lipoprint LDL Profile Versus Traditional Lipid Profile

- After obtaining informed consent, serum samples were obtained from 273 self-declared healthy individuals between the ages of 18 and 85 years. Study participants consisted of 166 female and 107 male of which 47% were Caucasian, 18% Hispanic, 16% Asian, 5% African American and 14% undeclared.
- Total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) were measured on a Dimension Instrument clinical analyzer (Dade Behring) using Dade Behring reagents.
- Subjects were segregated into two groups, “normolipidemic” and “dyslipidemic” based on ATP III desirable lipids status (total cholesterol, triglycerides, LDL-C and HDL-C).
- The Lipoprint LDL system and LDL Subfractions kit (Quantimetrix Corporation, Redondo Beach, CA) was used to measure the cholesterol levels in all the lipoproteins subfractions, that is: large VLDL, Mid-C (VLDL remnants), Mid-B (large IDL), Mid-A (small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 to LDL-7 (small dense LDL) and HDL in both, the “normolipidemic” and “dyslipidemic” groups.
- The Lipoware software automatically calculates the amount of cholesterol in each lipoprotein subfraction based on the total cholesterol of the sample and determines if the subfraction cholesterol values are within the established reference ranges for each subfraction.
- Results obtained from the traditional lipid profiles were compared to the Lipoprint LDL subfraction profiles to calculate the number of discordant profiles.

Lipoprint LDL Profile Versus Traditional Lipid Profile
A second set of 22 serum samples from 9 males and 13 females was obtained. The 10-year CVD risk assessment for each sample was calculated using the new ACC/AHA guidelines ASCVD risk calculator. The 10-year risk assessment results obtained by the ASCVD risk calculator were compared to other risk assessment methods listed below.

- **ASCVD Risk Calculator –** The samples were analyzed using the ASCVD 10-year risk calculator and classified for CAD risk according to the ACC/AHA guidelines criteria.
- **Lipoprint LDL Subfractions –** The samples were also analyzed for LDL subfraction using the Lipoprint LDL method and were classified for CAD risk according to the Lipoprint LDL system reference ranges.
  - **VLDL-C –** The samples were analyzed for VLDL cholesterol derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 22 mg/dL, Borderline High: 23-30 mg/dL, High: > 30 mg/dL).
  - **Mid-C –** The samples were analyzed for Mid-C (VLDL remnants cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 23 mg/dL, Borderline High: 24-26 mg/dL, High: > 26 mg/dL).
  - **Mid-B –** The samples were analyzed for Mid-B (IDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 15 mg/dL, Borderline High: 16-18 mg/dL, High: > 18 mg/dL).
  - **Mid-A –** The samples were analyzed for Mid-A (small IDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 25 mg/dL, Borderline High: 26-28 mg/dL, High: > 28 mg/dL).
  - **LDL-1 –** The samples were analyzed for LDL-1 (large buoyant LDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 57 mg/dL, Borderline High: 58-60 mg/dL, High: > 60 mg/dL).
  - **LDL-2 –** The samples were analyzed for LDL-2 (small dense LDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 30 mg/dL, Borderline High: 31-33 mg/dL, High: > 33 mg/dL).
  - **LDL-3 –** The samples were analyzed for LDL-3 (small dense LDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: ≤ 6 mg/dL, Borderline High: 7-9 mg/dL, High: > 9 mg/dL).
  - **LDL-4 to LDL-7 –** The samples were analyzed for LDL-4 to LDL-7 (small dense LDL cholesterol) derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Desirable: 0 mg/dL, High: ≥ 1 mg/dL).
  - **HDL-C –** The samples were analyzed for HDL cholesterol derived from the Lipoprint test and classified for CAD risk according to the Lipoprint HDL system reference ranges (Desirable: ≥ 50 mg/dL, Borderline Low: 40-49 mg/dL, Low: < 40 mg/dL).
- **NMR LDL Particle Number (LDL-P) –** The samples were independently analyzed for LDL-P by the NMR method and were classified for CAD risk based on the NMR method reference ranges (Low: < 1000 nmol/L, Moderate: 1000-1299 nmol/L, High: > 1300 nmol/L).

Other CAD Risk Assessment Methods

- **Total Cholesterol –** The samples were analyzed for total cholesterol and classified for CAD risk according to the ATP III guidelines reference ranges (Desirable: < 200 mg/dL, Borderline High: 200-239 mg/dL, High: ≥ 240 mg/dL).
- **LDL-C –** The samples were analyzed for LDL cholesterol (LDL-C) as derived from the Lipoprint test and were classified for CAD risk according to the ATP III guidelines (Desirable: < 100 mg/dL, Average: 100-129 mg/dL, Borderline High: 130-159 mg/dL, High: ≥ 160 mg/dL).
- **Non-HDL-C –** The samples were analyzed for non-HDL cholesterol derived from the Lipoprint test and classified for CAD risk according to the ATP III guidelines reference ranges (Desirable: ≤ 22 mg/dL, Borderline High: 23-30 mg/dL, High: > 30 mg/dL).

- **HDL-C –** The samples were analyzed for HDL cholesterol (HDL-C) as derived from the Lipoprint test and were classified for CAD risk according to the ATP III guidelines (Desirable: ≥ 50 mg/dL, Borderline Low: 40-49 mg/dL, Low: < 40 mg/dL).
- **TC/HDL-C –** The samples were analyzed for TC/HDL-C ratio derived from the Lipoprint test and classified for CAD risk according to the ATP III guidelines reference ranges (Desirable: < 4.0, Average: 4.0-5.0, Borderline High: > 5.0 ≤ 6.0, High: > 6.0).
- **LDL-C/HDL-C –** The samples were analyzed for LDL-C/HDL-C ratio derived from the Lipoprint test and classified for CAD risk according to the ATP III guidelines reference ranges (Desirable: < 3.0, Average: 3.0-4.0, Borderline High: >4.0 ≤ 6.0, High: > 6.0).
- **Average LDL Particle Size –** The samples were analyzed for average LDL particle size derived from the Lipoprint test and classified for CAD risk according to the Lipoprint LDL system reference ranges (Type A: ≥ 269 Å, Intermediate: > 265 < 268 Å, Type B: 30 ≤ 265 Å).

Results

Traditional Lipid Profile Versus Lipoprint LDL Profile

- Serum samples from 273 self declared normal individuals were assayed for the traditional lipid risk factors. The test results, (mean and range) for the study subjects are: total cholesterol 196 (104 – 319) mg/dL, triglycerides 96 (25 – 345) mg/dL, LDL-C 117 (58 – 215) mg/dL and HDL-C 55 (26 – 137) mg/dL.
- Out of the 273 total subjects, 132 (48%) were classified as the normolipidemic population according to the ATP III lipid guidelines while 141 (52%) had at least one parameter outside the ATP III recommendations and were classified dyslipidemic population.
- The samples were also tested using the Lipoprint LDL Subfractions Kit. The test separates up to 12 serum lipoprotein fractions and subfractions. VLDL – the largest particles – remain at the top of the separating gel followed by Mid-C (VLDL remnants), Mid-B and Mid-A (large and small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 through LDL-7 (small dense LDL) and HDL (see Figure 1).
- The cholesterol concentration of each lipoprotein fraction and subfraction was calculated using the Lipoprint System Lipoware analysis software based on the measured relative area of each lipoprotein band and the total cholesterol of the sample. A color-coded profile containing the cholesterol measurement for all the lipoprotein fractions and subfractions was generated for each individual sample (see Figures 1 and 2).
- The test results of the 273 samples obtained by the traditional lipid profile were compared to the test results obtained by the Lipoprint LDL test method. Of this total, 132 samples were classified normolipidemic according to the ATP III guidelines, based on the Lipoprint test results, 18 (14%) were discordant and excluded because the Lipoprint results for a given LDL subfraction were outside the 95% confidence interval (mean ± 2SD) for the given subfraction. The remaining 114 samples were classified normal according to the Lipoprint LDL test profile.
- Of the 273 total samples, the remaining 141 samples had one or more lipid parameters outside the normal ATP III results of the 141 dyslipidemic were compared to the results obtained by the Lipoprint LDL method. The methods' comparison revealed that 69 (49%) out of the 141 dyslipidemic samples had a normal Lipoprint LDL subfraction distribution profile while the remaining 72 (51%) samples had abnormal profiles by both methods. These results clearly point out a high degree of risk assessment discordance between the traditional lipid profile and the Lipoprint methods. To clarify this discordance, a comparison study of CVD risk assessment methods was conducted as described below.

Comparison of CVD Risk Assessment By Various Test Methods

In order to determine how different CVD risk assessment methods compare to each other at the individual patient level, serum samples from 22 random individuals, 9 Males and 13 females, were obtained and tested and analyzed for CVD risk by various traditional and non traditional test methods listed below. The individual patient's risk classification by each method is listed in Table 1. The percentages of CVD risk classification by method are shown in Figure 2.

- NCEP ATP III Guidelines (LDL Cholesterol) – For years, LDL-C has been the primary target for CAD treatment as stated by the NCEP ATP III guidelines and has been the primary lipid criteria for most demographic and outcome based studies used to assess CVD risk. LDL levels less than 100 mg/dL are considered normal or desirable, however, it is well documented that residual risk may persist even at optimal LDL-C levels less than 70 mg/dL while other individuals are CVD risk free even at very high LDL-C levels.

  The LDL-C range for all the samples tested was between 101 mg/dL and 169 mg/dL. Using a LDL-C cutoff of 160 mg/dL, 3 (13.6%) of the 22 individuals had elevated LDL-C and were classified high CVD risk.

  Nine (40.9%) individuals had LDL-C levels between 130 mg/dL and 159 mg/dl and were classified above average risk. The remaining 10 (45.5%) individuals LDL-C between 100 mg/dL and 129 mg/dL and were classified average risk. None had desirable LDL-C below 100 mg/dL.
- ACC/AHA Guidelines (ASCVD Risk Calculator) – The 10-year ASCVD risk for each of the 22 test samples was estimated using the ACC/AHA ASCVD risk calculator as recommended by the new guidelines. The parameters used by the ASCVD risk calculator include Sex, Age, Race, Total Cholesterol, HDL-Cholesterol, Systolic Blood Pressure, treatment for High Blood Pressure, Diabetes and Smoker. Samples with a10-year ASCVD risk less than 7.5% were classified desirable while samples with a 10-year ASCVD risk 7.5% or higher were classified as high risk.

  Based on the ASCVD risk calculator, a total of 7 (31.8%) individuals were identified as High Risk. Six (27.3%) of the individuals were classified high risk due to none lipid risk factors such as hypertension, diabetes or advanced age. Only one (4.5%) individual were classified high risk based on dyslipidemia alone. Fifteen (68.2%) individuals had a 10-year ASCVD risk lower than 7.5% and were classified desirable.
- Lipoprint LDL Subfractions – The samples were also tested using the Lipoprint LDL method to measure the amount of cholesterol in all the individual lipoprotein fractions and subfractions, that is: VLDL, Mid-C (VLDL remnants), Mid-B (large IDL), Mid-A (small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 to LDL-7 (small dense LDL) and HDL. Based on the Lipoprint LDL normal reference ranges, test results showed that increased levels of Mid-C (VLDL remnants), Mid-B (IDL) and LDL-3 or higher (small dense LDL) appear to be indicators of CVD risk. The large buoyant LDL subfractions Mid-A and LDL-1 appear not to be CVD risk indicators at all.

  After measuring the cholesterol levels in all the lipoprotein fractions and subfractions a total of 9 (40.9%) individuals were classified as high risk based on increased cholesterol levels of one or more atherogenic LDL subfraction. Of these high risk individuals, 7 (31.8%) had elevated levels of small dense LDL-3 and 5 (22.7%) had elevated Mid-B (IDL-C). An additional 6 (27.3%) individuals were classified borderline high due to slight increases in IDL or small dense LDL. Ten (45.0%) individuals had HDL levels below the desirable cutoff of 40 mg/dL. Seven (31.8%) individuals had a normal distribution of the LDL subfractions even though some had elevated triglycerides and or low HDL-C.
- Lipoprint LDL Average Particle size – The Lipoprint LDL method was used to estimate the LDL average particle size for each sample. LDL average particle sizes greater than 268 Å were classified Type A or predominantly large buoyant LDL. LDL average particle sizes between 265 Å and 268 Å were classified Intermediate and less that 265 were classified Type B or small dense LDL.

  Based on the Lipoprint LDL Mean Particle Size, 7 (31.8%) individuals were classified high risk Type B due to low average LDL particle size. These individuals correspond to the same 7 individuals with increased levels of small dense LDL. An additional 7 (31.8%) individuals were classified borderline risk due to their intermediate mean LDL particle size. The remaining 8 (36.4%) were classified low risk or Type A.
- NMR LDL Particle Number – The samples were also independently tested for LDL particle number (LDL-P) using the Nuclear Magnetic Resonance (NMR) method. Samples with LDL-P less than 1000 were classified low risk. Samples with LDL-P 1000 to 1299 were classified moderate risk and 1300 and higher were classified high risk.

  Sixteen (72.7%) of the 22 samples tested were classified at high risk for CVD by the NMR particle number (LDL-P) method. Five (22.7%) additional samples were classified moderate risk for a total of 95.5% of the 22 individual classified as moderate to high risk. Only one (4.5%) of the 22 samples was classified low risk.

Other CAD Risk Assessment Methods

In addition to the above described methods, the levels of Total Cholesterol, Triglycerides, Non-HDL-Cholesterol, the Total Cholesterol to HDL-Cholesterol ratio (TC:HDL-C ratio), LDL-Cholesterol to HDL-Cholesterol ratio (LDL-C:HDL-C ratio) were also determined.

- **Total Cholesterol –** Total cholesterol is the most frequently used test to identify dyslipidemias, however its CVD risk predictive value is very limited. The total cholesterol for the 22 samples tested ranged between 169 mg/dL and 238 mg/dL with a mean value of 207 mg/dL. Using the ATP III suggested desirable level of less 200 mg/dL, 12 (54.5%) of the 22 samples qualified as borderline high risk and the remaining 10 (45.5%) had desirable Total cholesterol levels.
- **Triglycerides –** The utility of triglycerides testing as a predictor of CVD has always been controversial, however, their influence in atherogenic dyslipidemia has gained recognition as a significant risk factor in diabetes and CVD. Based on ATP III desirable triglyceride levels 7 (31.8%) of the 22 samples had high triglycerides over 200 mg/dL and 5 (22.7%) of the samples had borderline high triglyceride between 150 mg/dL and 200 mg/dL. The remaining 10 (45.5%) samples had desirable triglyceride levels of less than 150 mg/dL.
- **Non-HDL Cholesterol –** Non-HDL cholesterol has been considered an alternative to LDL-C for the assessment of CVD risk. Results from this evaluation found very little difference between the two methods. Three (13.6%) of the 22 samples had Non-HDL-C greater that 190 mg/dL and were classified high risk. Ten (45.5%) had Non-HDL-C between 160 mg/dL and 189 mg/dL and were classified borderline high. Eight (36.4%) had Non-HDL-C 130 mg/dL and 159 mg/dL and were classified average risk and one (4.5%) had Non-HDL-C less than 139 mg/dL and was classified desirable.
- **Total Cholesterol to HDL Cholesterol Ratio –** The total cholesterol to HDL cholesterol ratio is very commonly used to assess CVD risk. Using the ATP III recommended high CVD risk cutoff of greater than 6.0 for the TC:HDL-C ratio, 5 (22.7%) samples were classified high risk. Eight (36.4%) samples had TC:HDL-C ratios between 5.0 and 6.0 and were classified borderline high. Five (22.7%) samples had TC:HDL-C ratios between 4.0 and 5.0 and were classified average risk and four (18.2%) had TC:HDL-C ratio of less than 4.0 and was classified desirable.
- **LDL Cholesterol to HDL Cholesterol Ratio –** LDL cholesterol to HDL cholesterol ratio is very commonly used to assess CVD risk. Using the ATP III recommended high CVD risk cutoff of greater than 6.0 for the LDL-C:HDL-C ratio, only one (4.5%) sample was classified high risk. Three (13.5%) samples had TC:HDL-C ratios between 4.0 and 6.0 and were classified borderline high. Eleven (50.0%) samples had TC:HDL-C ratios between 3.0 and 4.0 and were classified average risk and 8 (31.8%) had TC:HDL-C ratio of less than 3.0 and was classified desirable.

Discussion

Coronary artery disease (CAD) continues to be the leading cause of death in most of the developed countries and much of the developing world in spite of more risks awareness and increased treatment of individuals at risk. The role of lipids and lipoproteins in atherogenesis has been demonstrated by numerous studies but the methods for assessing CAD risk have continued to be the subject of much debate. Until recently, LDL cholesterol (LDL-C) was accepted as the primary target for CAD treatment. In November 2013, the ACC/AHA Task Force released new guidelines and a new CVD risk calculator based on data from randomized control trials. The risk calculator weighs heavily on non-lipid risk factors reducing the role of LDL-C and other lipid measurements as risk factors.

Recent clinical studies suggest that blood cholesterol and LDL-C levels as currently measured may not reflect the actual risk of CAD since many individuals that develop CAD have the same levels as those that do not develop CAD. These findings suggest that dyslipidemia, as currently defined, may not be a good indicator of CAD risk. The most common lipid disorder associated with CAD is a pro-atherogenic dyslipidemia characterized by the presence of highly atherogenic small dense LDL particles and or intermediate density lipoproteins and VLDL remnants particles that may not be reflected by the total cholesterol or LDL-C measurements. This pro-atherogenic dyslipidemia can be best identified by measuring all the individual atherogenic and non-atherogenic LDL particles in the individual's sample.

In this study, 22 randomly selected individuals were tested for CVD risk using the new ASCVD risk calculator. In addition, the samples were tested by other traditional and non-traditional test methods such as LDL-C, NMR LDL particle number, total cholesterol, triglycerides, non-HDL cholesterol, total cholesterol to HDL-C ratio and LDL-C to HDL-C ratio. These are methods that do not discriminate atherogenic from non-atherogenic LDL particles. The samples were also analyzed using the Lipoprint LDL subfractions test system to measure the cholesterol in each individual LDL subfractions as well as mean LDL particle size. Results for each of the 22 individuals are shown in Table1 based on each of the 9 different test methods. The individual samples were classified according to the CVD risk from High risk to Desirable. The percentage of samples at the various levels of risk according to each test method is shown in Figure 2.

The results of this study indicate that the number of individuals classified as moderate or high risk of CVD varies with the test method used. The highest number of individuals classified moderate or high risk was 21 (95.4%) out 22 based on the NMR LDL particle number. Of the 21 high risk individuals, 9 were classified high risk by the NMR particle number method only suggesting that NMR may overestimate risk. The lowest number of borderline high or high was 4 (18%) based on the LDL-C to HDL-C ratio.

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According to the ASCVD risk calculator, 7 (31.8%) of the 22 individuals were at high risk of CVD. Five of the seven samples were classified high risk based on non-lipid risk factors such as hypertension, diabetes and advanced age and only 2 were classified as the results of dyslipidemia. Based on the Lipoprint LDL subfractions, 15 (68.2%) of the individuals were classified intermediate or high risk. Interestingly, each of the 9 samples classified high risk by Lipoprint was also classified high risk by one or more other method. The borderline high and high risk individuals varied between 54% and 59% according to the other test methods. The findings of this study suggest that the ASCVD risk calculator appears to underestimate the risk associated with dyslipidemias when compared to other methods while the Lipoprint LDL subfractions test appears to capture all the high risk individuals classified as high risk by all the other methods. Considering the fact that CVD is a life long progressive disease, early recognition is very important in primary prevention and treatment. Use of the Lipoprint LDL test to measurement of the triglyceride enriched VLDL remnants, IDL and small dense LDL subfractions could provide early identification of individuals at risk for CVD.

Conclusion

Results from this study suggest the following points:

- The traditional lipid profile including total cholesterol, triglycerides, LDL-C and HDL-C do not always appear to discriminate between individuals at increased risk of CVD from low risk individuals especially among borderline high individuals.
- An individual's risk classification may vary with the test method used. Some methods classify most at risk samples while others classify very few at risk samples.
- Measurement of small dense LDL, VLDL remnants and IDL using the Lipoprint LDL subfraction test identified all the individual classified as high CVD risk from all the other test methods combined.
- Large bouyant LDL cholesterol as measured by the Lipoprint LDL Subfractions Test was not associated with increased CVD risk.
- The NMR LDL-P method classified 72.7% of the test samples as high risk which accounted for approximately 100% more than the other test methods.
- In the studied population, non traditional lipid testing methods such as total cholesterol to HDL-C ratio and triglycerides appeared to be better CVD risk indicators than the traditional lipid profile based on LDL-C.
- The new ACC/AHA guideline's recommendations are very useful for preventing CVD events in individuals with existing CVD and diabetes; however, they appear to be less helpful in identifying CVD risk in younger and older individuals in primary prevention.
- The Lipoprint LDL subfractions test identified all the individuals classified as high risk by the other test methods other than NMR LDL-P that identified twice as many high risk individuals than all the other test method.
- This study suggests that the Lipoprint LDL test appears to identify individuals at risk for CVD due to dyslipidemia in both the young and the old and could be helpful in primary prevention in individuals not recognized by the new ASCVD risk calculator.

Figure 1: Lipoprint LDL Profile

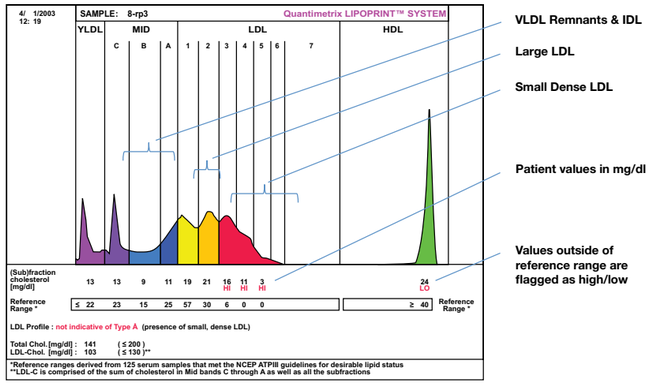

Figure 2: CVD Risk Assessment Methods Comparison

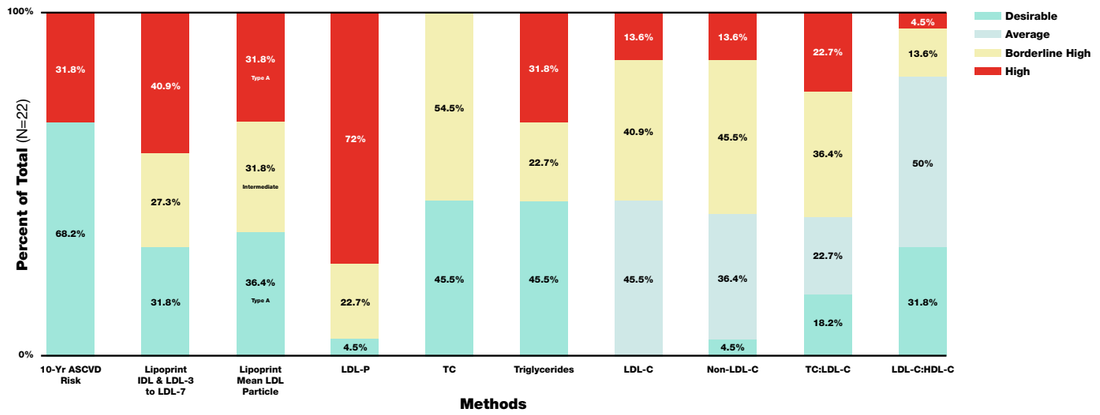

Table 1: CVD Risk Data

Sample ID	ACC/AHA 10-Year ASCVD Risk %	Lipoprint VLDL [mg/dL]	Lipoprint IDL-C [mg/dL]	Lipoprint IDL-B [mg/dL]	Lipoprint IDL-A [mg/dL]	Lipoprint LDL-1 [mg/dL]	Lipoprint LDL-2 [mg/dL]	Lipoprint LDL-3+ [mg/dL]	Lipoprint HDL-C [mg/dL]	Lipoprint Mean LDL Particle Size Å	NMR LDL-P (nmol/L)	ATP III TC [mg/dL]	ATP III TRIGS [mg/dL]	ATP III LDL-C [mg/dL]	ATP III Non-HDL [mg/dL]	ATP III TC:HDL-C Ratio	ATP III LDL-C: HDL-C Ratio
002	8.6	49	26	23	14	23	31	20	38	258	1281	235	471	147	197	6.2	3.9
005	17.7	28	24	20	25	52	40	6	43	269	1761	238	103	166	195	5.5	3.9
006	4.8	33	19	18	22	37	49	21	26	263	2425	228	186	169	202	8.8	6.5
008	1.8	43	23	17	12	18	25	12	33	262	1152	185	321	108	152	5.6	3.3
009	8.6	43	29	20	15	23	26	17	35	260	1376	215	245	136	180	6.1	3.9
010	3.6	42	22	19	13	20	25	22	27	254	1772	208	284	139	181	7.7	5.1
011	4.4	22	23	15	18	46	40	7	50	268	1936	222	115	149	172	4.4	3.0
012	7.1	35	21	23	27	43	32	6	37	268	1843	223	139	150	186	6.0	4.1
016	1.7	28	20	15	16	45	46	9	37	266	2038	217	153	152	180	5.9	4.1
017	1.6	31	20	14	20	54	31	2	48	270	1641	220	116	141	172	4.6	2.9
022	1.7	23	21	16	23	58	43	5	48	269	1980	237	101	165	189	4.9	3.4
023	5	36	20	12	14	37	18	0	31	272	1236	169	173	101	138	5.5	3.3
026	11.9	26	17	14	20	41	30	4	45	269	1690	196	105	125	151	4.4	2.8
028	5.7	43	21	13	9	22	33	14	30	262	1723	186	219	114	156	6.2	3.8
029	21.8	36	22	16	15	30	32	11	33	265	1445	196	196	127	163	5.9	3.8
030	0.6	33	21	14	20	55	27	2	54	271	1656	226	169	139	172	4.2	2.6
031	4.2	17	14	13	28	42	11	0	54	274	1299	180	55	108	126	3.3	2.0
034	9.7	22	14	13	16	32	26	7	51	266	1283	183	109	109	132	3.6	2.1
036	0.5	21	13	15	27	51	8	0	60	275	1484	196	71	115	136	3.3	1.9
037	4.5	44	22	13	11	27	30	7	32	266	1375	186	233	111	154	5.8	3.5
039	1.9	22	15	16	21	49	45	8	40	267	1998	216	83	153	176	5.4	3.8
040	18.8	42	22	13	11	30	25	5	51	267	939	199	287	107	148	3.9	2.1
	< 7.4 % Desirable	≤ 22	≤ 23	≤ 15	≤ 25	≤ 57	≤ 30	≤ 6	≥ 50 High	≥ 269 Type A	< 1000 Low	< 200 Desirable	< 150 Desirable	< 100 Desirable	< 130 Desirable	< 4.0 Desirable	< 3.0 Desirable
									40-49					100 - 129 Average	130 - 159 Average	4.0 - 5.0 Average	3.0 - 4.0 Average
		23-30	24-26	16-18	26-28	58-60	31-33	7-9		> 265 ≤ 268 Intermediate	1000-1299 Moderate	200 - 239 Borderline high	> 150 ≤ 199 Borderline high	130 - 159 Borderline high	160 - 189 Borderline high	> 5.0 - 6.0 Borderline high	> 4.0 ≤ 6.0 Borderline High
	≥ 7.5 % High	> 30	> 26	> 18	> 28	> 60	> 33	> 9	< 40 Low	≤ 265 Type B	> 1300 High	≥ 240 High	≥ 200 High	≥ 160 High	≥ 190 High	> 6.0 High	> 6.0 High

Quantimetrix