

A Novel Approach to Measure Apolipoprotein B/Apolipoprotein AI Ratio Using the Vertical Auto Profile Method

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Abstract

Several studies have shown that Apolipoprotein (Apo) B/Apo AI ratio is an important indicator of cardiovascular risk, especially in insulin resistant subjects. However, Apo B and Apo AI are not routinely measured due to the added cost. Our objective was to provide Apo B/Apo AI ratio with no additional cost by developing a statistical model for calculating Apo AI based on Vertical Auto Profile (VAP) ultracentrifuge cholesterol measurements and utilizing it with Apo B calculated by a model previously published by us. A formula to calculate Apo AI was developed by stepwise forward multiple linear regression using lipoprotein cholesterol classes and subclasses values in 204 serum samples measured by VAP. Only high density lipoprotein₃ (HDL₃), HDL₂, and very low density lipoprotein contributed significantly ($r = 0.93$). The formula was verified by comparing with direct Apo AI using 1058 serum samples. This comparison yielded an r of 0.92 with a standard error of estimate (s.e.) of 10.3 mg/dl, and 54%, 72%, 85%, 96%, and 99% of patients had calculated Apo AI within (\pm) 5%, 7.5%, 10%, 15%, and 20%, respectively, of direct values. To derive and compare calculated Apo B/Apo AI ratio with direct ratio, Apo B was measured using 842 of 1,058 samples. This ratio comparison yielded an r of 0.96 and s.e. of 0.05, suggesting an excellent agreement between calculated and direct Apo B/Apo AI ratios. These results suggest that Apo B/Apo AI ratio can be reliably determined using VAP cholesterol measurements with no additional cost.

Background

Apolipoproteins are key components of lipoprotein particles and play important roles in their synthesis and transport, binding to specific receptors for clearance, and activation or inhibition, of key enzymes involved in metabolism. Apolipoprotein AI (Apo AI) is the major component of high density lipoprotein (HDL) constituting 45% of its molecular mass, and is responsible for its anti-atherogenic property by acting as a cofactor for the enzyme lecithin cholesterol acyl transferase (LCAT) and as a mediator in transfer of cholesterol (c) from cells to HDL particles, which are key steps involved in reverse cholesterol transport. Apolipoprotein B100 (Apo B), on the other hand, is the major apolipoprotein of low density (LDL), intermediate density (IDL) and very low density (VLDL) lipoproteins, and is involved in their metabolism, including binding of LDL to specific receptors. While Apo B plays a significant role in delivering cholesterol to tissue cells thus acting as an atherogenic apolipoprotein; Apo AI plays a major role in reverse cholesterol transport, acting as an anti-atherogenic apolipoprotein. Measurement of Apo B represents total atherogenic particles in circulation because each atherogenic lipoprotein contains only one molecule of Apo B, whereas measurement of Apo AI represents total antiatherogenicity since Apo AI is responsible for reverse cholesterol transport. Therefore, a ratio of Apo B/Apo AI should provide a net balance between atherogenic and antiatherogenic lipoprotein particles.

Several large epidemiological and prospective studies have shown that Apo B/Apo AI ratio is indeed a very important risk factor for

coronary heart disease (CHD). In Apolipoprotein-related MORTALITY RiSk (AMORIS) study (1), a prospective study which followed 175,000 Swedish males and females for 98 months, Apo B/Apo AI ratio had a stronger relationship with CHD risk than any other lipid ratios including total cholesterol (TC)/HDL-C, LDL-C/HDL-C, or non-HDL-C/HDL-C. Other large prospective studies, such as INTERHEART (2), MONICA/KORA (3), and Quebec Cardiovascular Study (4) have also confirmed that Apo B/Apo AI ratio is an independent risk factor and superior to several conventional risk factors.

Metabolic syndrome is primarily characterized by insulin resistance and atherogenic dyslipidemia (HDL-C \leq 40 mg/dl, triglycerides $>$ 150 mg/dl, and elevated small, dense LDL particles, i.e., LDL phenotype B), subjects with this syndrome are at higher risk of CHD. As per findings from the Third National Health and Nutrition Examination Survey (NHANES III) (5) metabolic syndrome is present in approximately 23% of US population (47 million Americans as per 2000 census). NHANES III (6) has also shown that Apo B/Apo AI ratio is significantly higher in subjects with metabolic syndrome compared to subjects without metabolic syndrome (0.9 vs 0.69; $p < 0.0001$) and is an independent risk factor.

Thus, it is clear that routine measurement of Apo B/Apo AI ratio is highly beneficial, given the fact that nearly one-fourth of the US population has metabolic syndrome. However, currently Apo B and Apo AI are not routinely measured, despite their known importance in the assessment of CHD risk, because of additional cost involved in their assay. **Here we present a procedure to calculate Apo B/Apo AI ratio, at no additional cost to the patient, from lipoprotein cholesterol values routinely obtained from VerticalAuto Profile (VAP) method.**

1) Lancet. 2001;358:2026-33. 2) Lancet. 2004;364:937-52. 3) Eur. Heart J. 2005;26:271-8. 4) Circulation. 1996;94:273-8. 5) JAMA. 2002;287:356-9. 6) Am. J. Cardiol. 2006;98:1369-73. 7) J. Lipid Res. 1994;35:159-168. 8) Clin. Chem. 2007;53(S6):A41.

Methods

Lipoprotein Analysis: Cholesterol concentrations of lipoprotein classes [HDL, LDL-Real, LDL, IDL, VLDL, and Lp(a)] and their subclasses (including HDL2 and HDL3) were measured using single vertical spin density gradient ultra-centrifugation based Vertical Auto Profile (VAP) method at Atherotech laboratory (7).

Apolipoprotein Analysis: Direct Apo AI and Apo B were measured using immunoturbidimetric method using Abbott Diagnostics Architect/C8000 analyzer in serum specimens used for development and validation of formula to determine Apo AI by the VAP method.

Triglycerides: Triglycerides analysis was performed using Abbott Diagnostics Architect/C8000 analyzer.

Statistical Analysis: Statistical analysis was performed by calculating mean (mg/dl), SD, % bias, and ratios using Microsoft Excel. Comparison of results from two different methods or tests was performed using linear regression analysis using Microsoft Excel. Statistical models to calculate Apo AI from lipoprotein cholesterol values obtained from the VAP method were developed using Stepwise Forward multiple regression analysis using JMP Statistical Discovery™ from SAS.

Results

Calculation of Apo B/Apo AI Ratio by the VAP Method: Apo B/Apo AI ratios for all specimens were calculated by first individually calculating Apo B and Apo AI by VAP method. The procedure to calculate Apo B using VAP method has been previously described by us (8). The procedure to calculate Apo AI is described below.

Development of Formula for Calculating Apo AI by VAP Method: A formula to calculate Apo AI based upon Stepwise Forward multiple regression analysis model was first developed. Apo AI measured from Architect/C8000 chemistry analyzer (Abbott Diagnostics) using 204 patient serum specimens (training set) was used as dependent variable and cholesterol concentrations of various lipoprotein classes and subclasses obtained from VAP test (HDL, HDL2, HDL3, LDL-Real, Lp(a), IDL, LDL, VLDL, LDL peak max time, non-HDL, total cholesterol, and triglycerides) using the same 204 patient serum specimens as independent variables. The model with fewer numbers of independent variables which gave the highest R (correlation coefficient) was used to develop regression equation to calculate Apo AI. The model that fitted these criteria included HDL subclasses (HDL2 and HDL3) and VLDL-C with an R value of 0.93. Based upon this model the following formula was employed to calculate Apo AI for patient serum specimens used for further validation.

$$\text{VAP Calculated Apo AI} = [(2.4591 \times \text{HDL3}) + (0.611 \times \text{HDL2}) + (0.555 \times \text{VLDL}) + 33.75]$$

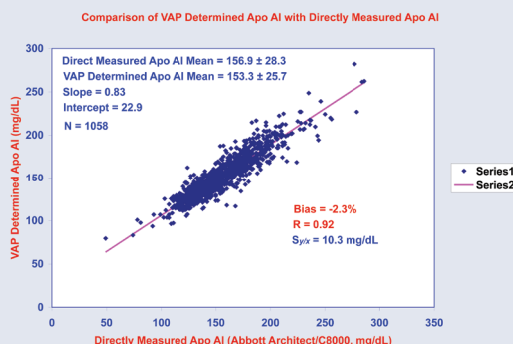
Validation of Formula: The above formula was verified by comparing VAP Calculated Apo AI with directly measured Apo AI using 1,058 fresh patient serum specimens (test set) with following results: Mean of measured Apo AI = 156.9 ± 28.3; Mean of VAP Calculated Apo AI = 153.3 ± 25.7; Bias = -2.3%; Slope = 0.83; Intercept = 22.9 mg/dl; R = 0.92; and S.E. = 10.3 mg/dl (see Figure 1).

We further evaluated accuracy of this procedure by calculating percentage of total patients tested who had VAP calculated Apo AI within a given bias range from measured Apo AI. 54%, 72%, 85%, 96%, and 99% of the tested patients were found to have VAP calculated Apo AI within (±)5%, 7.5%, 10%, 15%, and 20%, respectively (Table 1). The above results suggest a high accuracy of VAP calculated Apo AI.

VAP Apo B calculation: VAP Apo B was calculated using the formula previously published by us (8). Figure 2 shows comparison of VAP calculated Apo B with directly measured Apo B using 1,797 patients' serum specimens which also includes 842 patients serum used to calculate Apo B/Apo AI ratio in the present work.

VAP Apo B/Apo AI Ratio: VAP Apo B/Apo AI ratios for all specimens were calculated using Apo B and Apo AI calculated using VAP method described above and direct measured Apo B/Apo AI ratios were calculated using Apo B and Apo AI values obtained from direct measurement. Figure 3 shows comparison of VAP calculated Apo B/Apo AI ratios with directly measured ratios. The results suggest an excellent agreement between the two ratios, with a bias of only 2.6%. We have further confirmed these results using 147 patients attending clinic for lipid lowering therapy using IRB approved protocol.

Reproducibility of VAP Calculated Apo B/Apo AI Ratios: Within day and between day reproducibility of VAP calculated Apo B/Apo AI ratio is shown in Table 2. The data shown clearly suggest both an excellent short term and long term reproducibility of Apo B/Apo AI ratio calculated by the VAP method.



Comparison of VAP Calculated Apo B/Apo AI Ratio with Measured Ratio

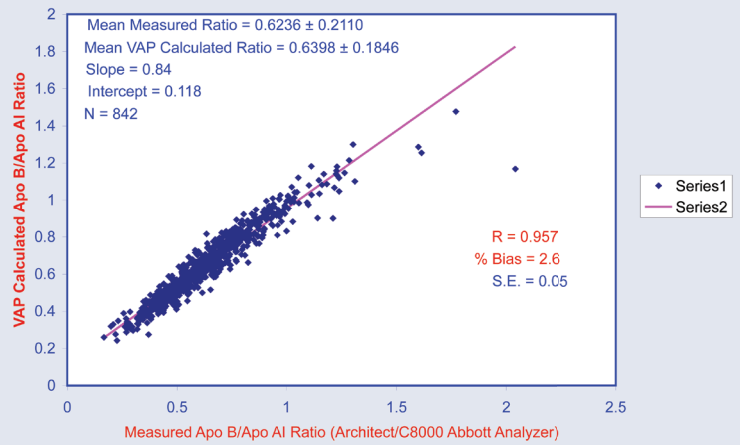


Table 1. Percent of Tested Patient Population Whose VAP Calculated Apo AI is Within a Given ± Percent (Bias) of Measured Apo AI

Bias Range of (VAP Calc. - Measured) Apo AI	Number of Patients	% of Total Patients (N = 1058)
±5%	570	53.9
±7.5%	756	71.5
±10%	898	84.9
±15%	1,016	96.0
±20%	1,045	98.8

Table 2: Within Day and Between Days Reproducibility of VAP Determined Apo B/Apo AI Ratio.

Day	Control 1 (Apo AI=132mg/dL; Apo B=81mg/dL)		Control 2 (Apo AI=147mg/dL; Apo B=118mg/dL)	
	N	%CV	N	%CV
1	126	3.0	121	2.5
2	163	2.7	167	2.6
3	158	2.8	159	3.0
4	131	3.7	132	2.6
5	102	2.8	104	2.6
Between (40) Days	4,700	3.0	4,454	2.9

Discussion and Conclusion

Several large prospective studies including AMORIS, INTER-HEART, Quebec Cardiovascular Study, and MONICA/KORA have clearly shown that Apo B/Apo AI ratio, which represents a balance of atherogenic and anti-atherogenic lipoproteins, is a superior marker of CHD risk to all conventional markers, including TC/HDL-C and LDL-C/HDL-C ratios. However, Apo B and Apo AI are not routinely measured, primarily due to the additional cost involved in their assay. Our data shown above clearly suggest that Apo B/Apo AI ratio can be accurately calculated using cholesterol concentrations of lipoprotein classes and subclasses as determined by the VAP method with no additional cost. We believe providing a superior CHD risk marker such as Apo B/Apo AI ratio at no additional cost will improve diagnosis and treatment of CHD.

1. Our results suggest that Apo B/Apo AI ratio, a superior CHD risk marker, can be accurately calculated from cholesterol concentrations of lipoproteins and their subclasses obtained from the VAP method, which is readily available.
2. VAP method provides Apo B and Apo AI and thus Apo B/Apo AI ratio at no additional cost.