Non-High-Density lipoprotein cholesterol or Apolipoprotein B in the prediction of myocardial infarction

Amit Kumar Pradhan[1], Ritabrata Mitra[2], Subinay Datta[1] and Mrinal Pal[1*]

[1]Department of Biochemistry, Burdwan Medical College, Near Baburbag Road, Burdwan-713104, West Bengal, India and [2]Department of Pulmonary Medicine, IPGME & R, SSKM Hospital, 244 AJC Bose Road, Kolkata-700020, West Bengal, India

Abstract: *Background:* It is well documented that elevated Low Density Lipoprotein cholesterol (LDL-C) is one of cardiovascular risk. However, not all patients of coronary heart disease possess elevated LDL-C level. There is a growing evidences that non-high density lipoprotein (non-HDL) cholesterol and Apo B carry on all of the potentially proatherogenic lipoproteins apart from LDL-C. The study was conducted to search the role of non-HDL(C) and ApoB as alternative to LDL-C for the better and useful predictor of myocardial infarction (MI). *Methods:* In this cross-sectional study, hundred patients of MI and 100 controls of age and sex matched were studied for LDL-C, non-HDL(C) and Apo B between March 2014 and April 2015 in the Department Cardiology and Biochemistry. *Result:* The data was analyzed using SPSS 11.5. Serum concentration of both non-HDL cholesterol and ApoB in cases is significantly higher than controls but no significant differences in concentration of serum LDL-C (Student's t test). On regression analysis it was shown that serum non-HDLC is better correlated with apo B than is LDL-C. On ROC curve analysis, it was found that non-HDL(C) had both the sensitivity and the negative predictive value 100% and 95.1%, whereas specificity and positive predictive value of Apo B were 96% and 94.6% respectively. On the other hand sensitivity and negative predictive value were 100% and 96.3%, whereas specificity and positive predictive value were 95% and 95.7% respectively. *Conclusion:* Both apoB and non–HDL-C was better predictor of MI than LDL-C and among these two, non-HDL(C) is much better.
Keywords: ApoB, Low Density Lipoprotein cholesterol, Myocardial infarction, Non-High Density Lipoprotein

Introduction

The Third Adult Treatment Panel (ATP III) of the National Cholesterol Education Program (NCEP) has recommended that elevated levels of low density lipoprotein cholesterol (LDL-C) is strongly associated with an increased risk for development of cardiovascular disease (CVD) and LDL-C is also recommended as primary target for lipid lowering therapy for prevention of CVD [1-2]. However, several studies have shown that significant numbers of CVD study population have normal LDL-C [3] and the cardiovascular benefits with statins may go beyond their influence on LDL-C levels [4]. Thus, LDL-C may not be the best lipid parameter to predict cardiovascular risk or to quantify the atheroprotective effect of statin therapy.

Two approaches have been proposed to provide a single measurement that includes all atherogenic lipoproteins. One is to measure the apolipoprotein B (apoB) concentration, which is a direct measurement of the concentration of proatherogenic particles, because each VLDL and LDL-C particle contain apoB [5]. Another lipid parameter has been proposed as alternative for LDL-C is Non-HDL(C) which is the difference between total and HDL-C. It represents cholesterol carry on all of the potentially proatherogenic apoB containing particles such as VLDL, IDL, LDL-C as well as chylomicron remnant and Lipoprotein (a) [6]. Thus the present study was conducted to search the role of non-HDL(C) and ApoB as alternative to LDL-C as a better and useful predictor of myocardial infarction (MI) and among Apo B and non- HDL(C) which is the better.

Material and Methods

Selection of subjects: Hundred patients irrespective of age and sex diagnosed as myocardial infarction (clinically and by ECG findings) were selected as case. All the cases

were positive to cardiac specific troponin I test. The selected patients were from Burdwan district and adjoining areas. The study was conducted between March 2014 and April 2015. The age and sex matched hundred controls were selected from the healthy persons from same region (Figure1). Both the cases and controls were selected by a simple random method. Risk factors of lipid metabolism disorder that were assessed including smoking, family history of myocardial infarction, age, blood pressure, and body mass index (BMI). Patients taking known lipid lowering medications or heparin, people with chronic kidney disease, and people experiencing a myocardial infarction within 3 months were not included in this study.

Fig-1: Study design of the present study
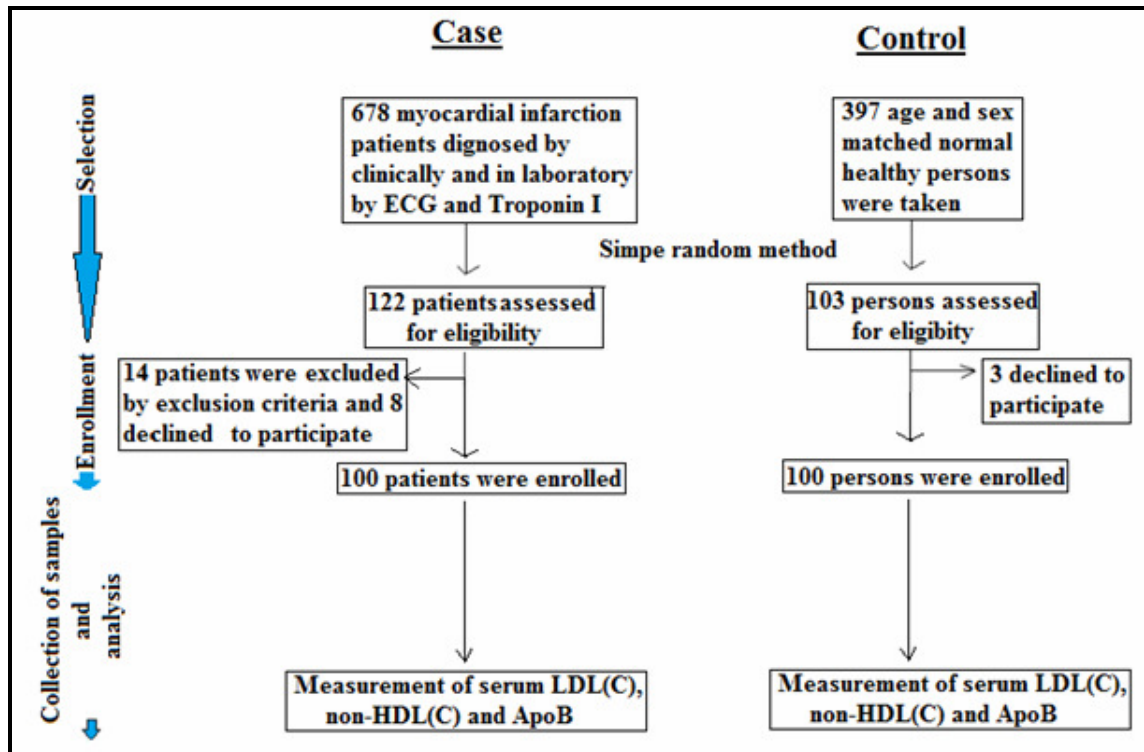
Study area: This hospital based cross-sectional study was conducted in the Cardiology clinics with the collaboration of Department of Biochemistry of Burdwan Medical College, Burdwan, and West Bengal, India.

Ethics Statement: The study was approved and permitted by the institutional ethics committee for care and use of laboratory and started after obtaining the written consent from the concerned ethics committee [Memo No.BMC/2179/1 (17)].

Collection of samples: Peripheral venous blood was drawn and allowed to coagulate at room temperature for 30-45 min, followed by centrifugation at 2500Xg for 15 min. Serum was separated and all assays were performed within 24 hours.

Parameters assay: Serum Total Cholesterol, LDL-C and HDL-C were measured by Cholesterol Oxidase–peroxidase (CHOD-PAP) [7] by a homogenous direct method from Genzyme Corporation, [8] by means of a direct enzymatic colorimetric assay, [9] and Triglyceride (TG) enzymatically with correction for endogenous glycerol [10] respectively. Intra-assay CV % of TC, TG, LDL-C and HDL-C were 1.2, 2.3, 2.8, 3.1 respectively. The inter-assay CV % of these parameters were 3.6, 4.1, 4.7 and 3.6 respectively. Non–HDL-C was calculated as total minus HDL-C [6]. All analysis was performed with autoanalyzer ERBA XL 600. HDL-C and LDL-C concentration were measured with the direct method using ERBA system packs. Total apoB100 was measured

by an immunoturbidimetric technique on the Hitachi 911 analyzer (Roche Diagnostics), with CVs of 5%.Using commercially available cardiac specific Trop I kit supplied by Zydus Company did troponin I test. It implies the principle of immunochromatography and the sensitivity of test is serum cardiac specific troponin I value at 0.5 ng/ml.

Statistical analysis: The data for biochemical analysis was subjected to standard statistical analysis such as Student's t test using the Statistical Package for Social Science (SPSS) 11.5 software. Variables were compared between cases and control subjects by using the Student's

unpaired *t* test. Associations between lipid marker levels were examined in subjects with myocardial infarction by Regression analysis. To obtain a better predictor of MI among non-HDL(C) and ApoB, ROC curve analysis was made. Data were expressed as mean values ± SD and $p < 0.05$ was considered as statistically significant.

Results

The characteristics of the study population: In the Table 1tudy groups has been shown and found that controls are age and sex matched with cases.

Table-1: Personal profile and clinical details of healthy persons and patients suffering from myocardial infarction			
	Healthy persons	**Patients suffering from myocardial infarction**	**p value**
Number of study population in each group	100	100	
Age	46.2 ± 7.6	45.9 ± 7.1	0.23
Sex			
Males	47	46	0.31
Females	53	54	
Demographic data			
Urban background	64	64	0.17
Rural background	36	36	
Qualitative serum cardiac specific troponin I test	Negative	Positive	

Comparison of serum LDL-C and non-HDL(C) between cases and controls – Unpaired t-test: The serum concentration of both non-HDL cholesterol and ApoB in cases of MI is significantly higher than controls (p = 0.00) but

differences in concentration of serum LDL-cholesterol between MI cases and controls are not significant (p = 0.115) (Table 2).

Table-2: Concentration of serum LDL-cholesterol and non-HDL-cholesterol in cases suffering from Myocardial infarction and Controls			
Category	**Concentration of serum LDL-C (mg/dl)**	**Concentration of serum non-HDL cholesterol (mg/dl)**	**Concentration of ApoB (mg/dl)**
IHD Cases (n=100)	104.30 ± 15.32	173.8 ±37.2	104.28 ± 22.3
Control (n=100)	101.60 ± 16.45	155.5 ± 33.4	94.61 α 19.92
IHD cases Vs Controls	p = 0.12	p = 0.00	p = 0.00
Values are mean ± SD; p > 0.05 not significant; p <0.05 significant			

Correlation of serum LDL-C, non-HDL(C) and ApoB in patients suffering from myocardial infarction-Regression analysis: Regression analysis 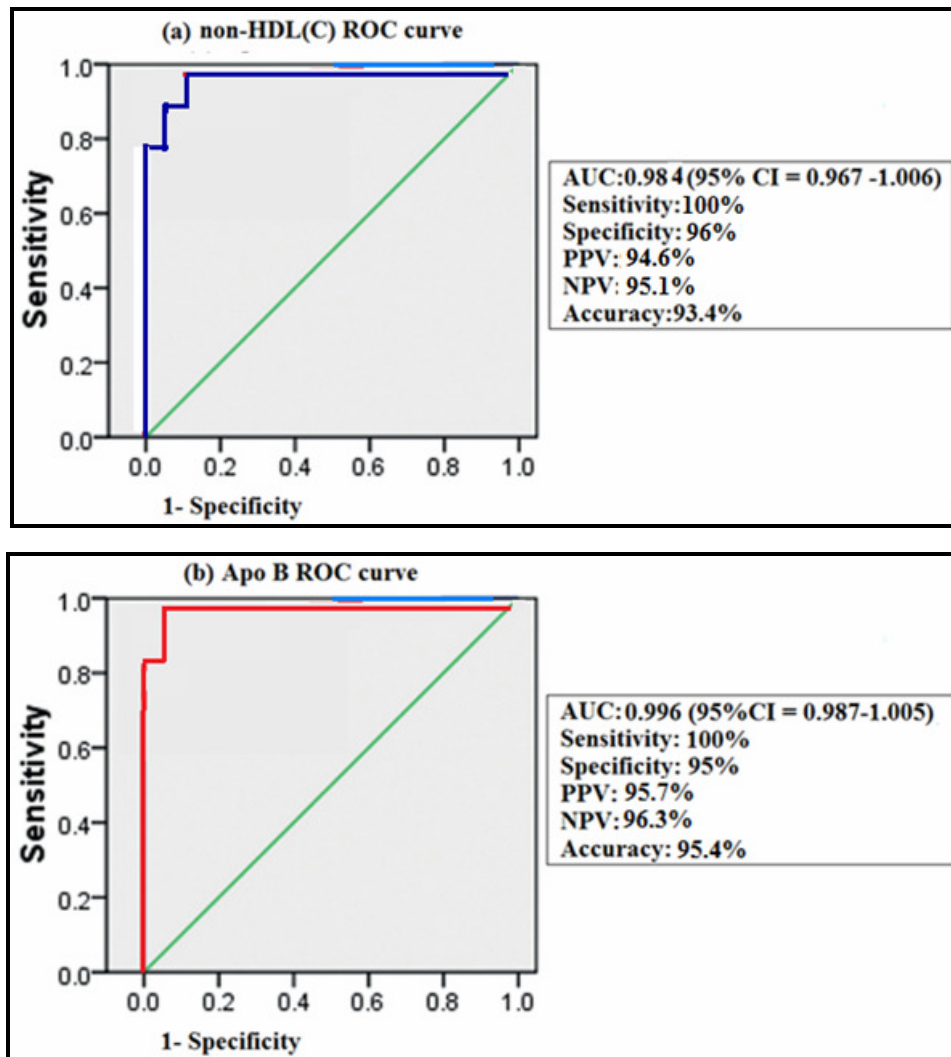was performed and it was shown that serum non–high-density lipoprotein cholesterol (non-HDLC) is better correlated with apo B than is LDL-C.

Table-3: Pearson's correlation between serum LDL-C, non-HDL(C) and ApoB in patients suffering from myocardial infarction

Category	r value	Significance
LDL-C vs non-HDL(C)	0.437	0.18
LDL-C vs ApoB	0.584	0.10
non-HDL(C) vs ApoB	0.931	0.02

Role of non-HDL(C) or ApoB in prediction of myocardial infarction-ROC curve: Both the sensitivity and the negative predictive value of non-HDL(C) were 100% and 95.1%, whereas specificity and positive predictive value were 96% and 94.6% respectively. Apo B had the sensitivity and the negative predictive value 100% and 96.3%, whereas specificity and positive predictive value were 95% and 95.7% respectively (Figure 2).

Fig-2: Receiver Operative Characteristic (ROC) curves of (a) non-HDL(C) and (b) Apo B for comparison of predictability of MI

(a) non-HDL(C) ROC curve

AUC:0.984 (95% CI = 0.967 -1.006)
Sensitivity:100%
Specificity: 96%
PPV: 94.6%
NPV: 95.1%
Accuracy:93.4%

(b) Apo B ROC curve

AUC:0.996 (95%CI = 0.987-1.005)
Sensitivity: 100%
Specificity: 95%
PPV: 95.7%
NPV:96.3%
Accuracy: 95.4%

Discussion

To assess whether serum LDL-C, non-HDL(C) or ApoB is the better and useful predictor of myocardial infarction it was found as some previous studies [3-4] that the serum concentration of LDL-C between MI cases and controls are not significantly different. As LDL-C is not the only lipoprotein involved in atherosclerotic heart disease but TG-rich VLDL and the so-called remnant lipoproteins are also atherogenic [11]. This is of particular importance when TG levels are high which is quite common for Individuals with abdominal obesity, metabolic syndrome or diabetic dyslipidmia. Despite their normal LDL-C, these patients produce highly atherogenic lipoproteins such as VLDL and IDL as well as small dense LDL-C particles. [12-14].

In the present study, the serum concentration of non-HDL cholesterol in cases of MI is significantly higher than controls. By subtracting HDL-C from the total cholesterol there is a measure of the amount of cholesterol that carries all lipoproteins except HDL-C.[6] Doing this simple mathematical calculation it will give the amount of cholesterol present within all lipoprotein that are atherogenic such as LDL-C, lipoprotein (a), IDL and VLDL remnant. [15] For LDL-C estimation, there are several limitations in their accuracy as it requires fasting samples and recommended against reporting a calculated LDL-C in patients who have fasting TG $\geq$ 400mg/dl or have type III hyperlipidemia [13].

Thus, in many cases of fasting hypertriglyceridemia such as in diabetes mellitus, there has no reliability to estimate LDL-C unless an immunoseparation technique for a direct LDL cholesterol determination is performed. However, comparison studies demonstrate that in some hypertriglyceridemic samples, a significant bias still exists with this method. [6] In contrast to the standard fasting lipid profile, non-HDL-C may be calculated on non-fasting specimens and may avoid the problem of calculating LDL-C with high TG, essentially making the need for a direct LDL-C assay obsolete. Non–HDL cholesterol thus represents a readily obtainable, inexpensive, and convenient measure of MI risk that may be superior to LDL cholesterol in many respects. In our analysis, patients of myocardial infarction have significantly higher apoB concentration than

controls, as one ApoB molecule per lipoprotein particle apoB reflects the total number of VLDL, IDL, and LDL-C particles and thus the concentration of all proatherogenic molecules [16]. Lipoproteins containing apoB must first enter the arterial wall and then undergo oxidative modification. This modification affects the structure of the apoB molecule such as the phospholipid membrane, yielding ligands for the scavenger receptors of macrophages in the arterial wall [17]. Subsequently, cholesterol accumulation and crystallization in macrophage cytoplasm leads to the formation of foam cells and progression to atherosclerotic plaque [18]. Another finding in present study was that serum LDL-C was not correlated both with non-HDL(C) and Apo B but serum non-HDLC is well correlated with Apo B in our study because both are good measure of β-lipoproteins.

So these results suggest that non-HDL(C) and Apo B are the as good as and in fact is better than LDL-C as a predictor of myocardial infarction. In a study by Cui et al non-HDL-(C) levels was good predictor of CVD mortality in both sexes [19]. In contrast, LDL-C which is the main focus of the NCEP guidelines was the weakest lipid predictor of CVD death in men and women. Kilgore et al estimated that 3.9 million US adult had high non-HDL (C) despite having a low LDL-C for which treatment initiation was recommended [20]. In a European cohort study showed that both non-HDL(C) and Apo B were strongly associated with risk of future CHD and had ability to predict its risk [21]. Even after treatment with statin who achieve low LDL-C level but have high concentration of Apo B and non-HDL(C) remains increased risk of cardiovascular diseases [17].

To find out the better predictor of MI among these two parameters, ROC curves analysis was done. On the basis of ROC curve the difference in AUC between Apo B and non-HDL(C) was about 0.012, which would translate into a true-positive frequency of about 1.2%. From this finding, it could lead to the conclusion that Apo B is a decidedly better predictor than non-HDL(C) in MI. But Apo B is superior to non-HDLC in a purely

statistical sense. But if these data turn out to apply to general populations for clinical purposes, it would be wise to use non-HDLC, because it is obtained from the standard lipid profile and not required any sophisticated instruments like nephelometer that is required for Apo B.

Conclusion

This study indicates that both ApoB and non–HDL-C is the better predictor than LDL-C.

Among Apo B and non- HDL(C), though Apo B is superior to non-HDL(C) to some extent but in developing countries where cost is the major hindrance, non-HDL(C) is the superior parameter for prediction of MI.

Acknowledgement

References

1. National Cholesterol Education Program Adult Treatment Panel III. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106(25):3143-421. *http://circ.ahajournals.rg /content/106/25/3143.citation*

2. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clerk LT, Hunninghake DB et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 2004; 110(2):227-39.

3. Datta S, Iqbal Z, Prasad KR. Comparison Between Serum hsCRP and LDL Cholesterol for Search of a Better Predictor for Ischemic Heart Disease. *Ind J Clin Biochem* 2011; 26(2):210-213.

4. Arsenault BJ, Boekholdt SM, Kastelein JJ. Lipid parameters for measuring risk of cardiovascular disease. *Nat Rev Cardiol.* 2011; 8(4):197-206.

5. Sniderman AD, Furberg CD, Keech A, Roeters van Lennep JE, Frohlich J, Jungner I, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. *Lancet.* 2003; 361(9359):777-780.

6. Hsia HS. Non-HDL Cholesterol: Into the Spotlight. *Diabetes Care* 2003; 26(1):240-242.

7. Bishop ML, Fody EP, Schoeff LE. Clinical Chemistry. 6th Edition, Philadelphia: *Wolters Kluwer Health,* 2010; 344.

8. Bishop ML, Fody EP, Schoeff LE. Clinical Chemistry. 6th Edition, Philadelphia: *Wolters Kluwer Health,* 2010; 348.

9. Bishop ML, Fody EP, Schoeff LE. Clinical Chemistry. 6th Edition, Philadelphia: *Wolters Kluwer Health*, 2010; 347.

10. Bishop ML, Fody EP, Schoeff LE. Clinical Chemistry. 6th Edition, Philadelphia: *Wolters Kluwer Health,* 2010; 345.

11. Bittner V. Non-HDL cholesterol: Measurement, interpretation and significance. *Adv Stud Med* 2007; 7(1):8-11.

12. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, et al. American Diabetes Association, American College of Cardiology Foundation. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care* 2008; 31:811-822.

13. Ginsberg HN, Stalenhoef AF. The metabolic syndrome: targeting dyslipidemia to reduce coronary risk. *J Cardiovasc Risk* 2003; 10(2):121-128.

14. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PWF et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation.* 2006; 113:20-29.

15. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-Analysis of the Relationship between Non–High-Density Lipoprotein Cholesterol Reduction and Coronary Heart Disease Risk. *J Am Coll Cardiol* 2009; 53:316-22.

16. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-High-Density Lipoprotein Cholesterol and Apolipoprotein B in the Prediction of Coronary Heart Disease in Men. *Circulation.* 2005; 112:3375-3383.

17. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ et al. Association of LDL Cholesterol, Non-HDL Cholesterol, and Apolipoprotein B Levels With Risk of Cardiovascular Events Among Patients Treated With Statins A Meta-analysis. *JAMA* 2012; 307(12):1302-1309.

18. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature.* 2010; 464(7293):1357-1361.

19. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, et al. Non-HDL lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. *Arch Intern Med* 2001; 161(11):1413-1419.

20. Kilgore M, Muntner P, Woolley JM, Sharma P, Bittner V, Rosenson RS. Discordance between high non-HDL cholesterol and high LDL-cholesterol among US adults. *Journal of Clinical Lipidology* 2014; 8:86-93.

21. Sondermeijer BM, Rana JS, Arsenault BJ, Shah PK, Kastelein JJ, Wareham NJ, et al. Non-HDL cholesterol vs. Apo B for risk of coronary heart disease in healthy individuals: the EPIC-Norfolk prospective population study. *Eur J Clin Invest* 2013; 43(10):1009-1015.

*All correspondences to: Dr. Mrinal Pal, Assistant Professor, Department of Biochemistry, Burdwan Medical College, Near Baburbag Road, Burdwan-713104, West Bengal, India. Email: mrinalpal77@rediffmail.com