[Frontiers In Bioscience, Landmark, 23, 458-463, January 1, 2018]

The "forgotten" modified lipoprotein subspecies

Ahmed Bakillah[1], Mohamed Zaiou[2]

[1]SUNY Downstate Medical Center, Department of Medicine, 450 Clarkson Ave, Brooklyn, NY-11203, USA, [2]Université de Lorraine, Faculté de Pharmacie, 5 Rue Albert Lebrun, 54000-Nancy, France

TABLE OF CONTENTS

1. Abstract
2. Background
3. Conclusion
4. Acknowledgments
5. References

1. ABSTRACT

Insights from preclinical and clinical studies have attempted to highlight the importance of modified lipoprotein particles in the pathogenesis of cardiovascular diseases (CVD). However, evidence is not conclusive. Since there is a relative dearth of clinical research in collecting useful information from traditional advanced lipoproteins testing, this present editorial introduces the aim of a special issue on modified lipoproteins as potential biomarkers for CVD. This issue aims at gathering a selection of insightful articles that address major challenges related to potential clinical use of modified lipoproteins as new CVD biomarkers. The editors seek to promote better mobilization of lipoproteins measurement for the pursuit of sustainable CVD clinical outcome and development of potential biomarkers. Knowledge and progress in this particular field will certainly help answering questions about clinical relevance of circulating modified lipoprotein subspecies and their potential use for better patient care and disease prevention. We hope that, when taken together, the focus on modified lipoproteins will stimulate new vision and reveal study key aspects for better clinical data outcome and more effective therapeutic strategies.

2. BACKGROUND

CVD is the leading cause of death and a major financial burden in most developed countries around the world, resulting in an estimated death toll of 17.5 million people in 2012 (~31% of all global deaths). Of these deaths, an estimated 7.4 million were due to coronary heart disease (CHD) and 6.7 million were due to stroke. The estimated annual cost of CHD and stroke for 2011–2012 was ~ $316.6 billion; and about 14% of the US population above 20 years of age has serum cholesterol higher than 240 mg/dL, a major risk factor for CVD. According to American Heart Association (AHA) policy statement, these costs will rise even more and will reach ~ $1 trillion annually in the United States by the year 2030, suggesting great need for powerful preventative measures (1).

During the past few decades, there have been significant advances in the treatment of some forms of CVD. In terms of CHD, remarkable research efforts have been made in lowering plasma cholesterol level by the widespread use of statins resulting in death rate reduction by ~30%-40%. In addition, human anti-PCSK9 antibodies (Alirocumab and Evolocumab) have been shown in a wide range of patients, such as in those with statins intolerance, to significantly decrease low density lipoprotein cholesterol (LDL) by ~50–70%. Multiple phase III studies with these new promising drugs were recently completed and both primary and secondary cardiovascular end points were successfully met with no new alarming safety signals (2). More recently, association studies using high-throughput genetics and genomics technology and newer genome-wide study approaches have sought to account for variation in risk factors hoping to identify relevant genetic markers for CVD. Despite all these efforts, CHD continue to be the leading cause of mortality worldwide. In addition, current approaches for the prevention and treatment of CVD are not fully effective in terms of risk reduction due to "residual risk" even when using a number of statin-combined therapeutic strategies. Thus, it has become obvious that validation of new biomarkers that may contribute to a better assessment of cardiovascular risk and help in implementing new powerful treatment strategies is of great importance in the current clinical research. In this context, the serum modified lipoproteins could be a good example of emerging paradigm for the discovery of novel biomarkers to define quite early CVD risk and pathophysiology.

Lipids hold a very important place as far as modifying vascular risk factors is concerned. In countries where there has been a significant reduction in CVD over the last four decades, reduction in blood pressure, lipids and smoking rates were the principal contributors (3). Total cholesterol and LDL-cholesterol (LDLc) values are two of the most important independent predictors of cardiovascular morbidity and mortality (4). A variety of lipoprotein assays have been developed that subfractionate different lipoprotein particles according to properties such as particle size, density, or charge. Advanced lipoprotein testing (ALT) have been proposed for improving assessment of cardiovascular risk and guiding lipid modifying therapies. These methods have mainly focused on the phenomenon of “atherogenic” and “ non-atherogenic” status of lipoproteins (LDL subclass phenotype A and LDL subclass phenotype B) after it was reported that ~ 80% of patients with an acute coronary syndrome or myocardial infarction showed normal plasma values of cholesterol, LDL and high density lipoprotein cholesterol (HDL). Great improvement have taken place in this filed and remarkable information was obtained from the assessment of atherogenic lipoprotein profile in serum of CVD patients by Lipoprint System Analysis leading to an important role of atherogenic lipoprotein spectrum in CVD outcome (5). However, information obtained by the Lipoprint Analysis and other analytical methods remained limited due to differences in clinical trial designs, studied populations, and primary outcomes rendering data inconsistent and difficult to interpret. Another important limitation could be related to differences in the nature and function of those isolated lipoprotein sub-fractions that are continuously target to various modifications during disease progression. Therefore, ALT (particle size, distribution and number) requires further deep understanding of the quality of different lipoprotein fractions by assessing their degree of alteration in order to gain full information for a better clinical outcome. Finally, promoting a transition toward more sustainable analysis of lipoprotein modification along with the existing ALT would be a beneficial task that may lead to better clinical outcome and patient management.

NMR spectroscopic analysis of sera from patients of large community-based study (Framingham Offspring Study) showed that small size LDL particles number is more strongly related to incident of CVD events than LDLc levels (6). In addition, results of other large-scale studies such as VA-HIT, MRFIT, HPS, MESA, and JUPITER trials revealed that elevated number of HDL particles predict better than HDLc the reduction of CVD risk and CHD death following multiple adjustments (7). However, there is as yet inconclusive data as to the extent to which lipoprotein subfraction measurements improve clinical assessment of CVD risk beyond standard lipid risk markers. Part of the solution to this issue may come from a more refined analysis of lipoproteins subspecies by using standardized and uniform analytical methodologies including NMR, molecular imaging, and ELISA techniques in well-powered clinical studies. These approaches may facilitate identification of a specific lipoprotein signature that is associated with lipid metabolism disorder and may lead to further improvements in CVD risk evaluation by discovery of novel biomarkers.

Atherogenic modification of LDL plays a crucial role in the pathogenesis of atherosclerosis, as modified LDL induces significant accumulation of cholesterol and lipids within the arterial wall (8). It is now well known that LDL particles undergo multiple modifications in human plasma including changes in size and density, acquisition of negative electric charge, oxidation, glycation, nitration, and carbamylation. In the past decades, oxidized LDL (oxLDL) has attracted attention as a blood marker that is associated with CHD. The oxidative modification of LDL in the artery wall has been implicated as one of the major physiologically relevant mechanisms for the pathogenesis of atherosclerosis (9, 10). In addition, there is widespread evidence supporting the role for lipid peroxidation in the molecular mechanism of the formation of the oxLDL as a pathogenic factor. Modified LDL are capable of inducing vascular inflammation through activation of innate immunity; thus, contributing to the progression of atherogenesis. In fact, the immunogenicity of modified LDL results in induction of self-antibodies specific to a certain type of modified LDL (11). These antibodies react with modified LDL forming circulating immune complexes which exhibit prominent immunomodulatory properties that influence atherosclerotic inflammation.

Since there is convincing evidence for direct involvement of non-enzymatic glycation of lipoproteins in the accelerated development of atherosclerosis in diabetic patients, most attention has been focused on the pathological properties of glycated lipoproteins. Glycation of LDL was found significantly increased in diabetic patients compared with normal subjects, even in the presence of good glycemic control (12). Metabolic abnormalities associated with glycation of LDL include diminished recognition of LDL by the classical LDL-receptor, and enhancement uptake of LDL by a low-affinity, high capacity receptor pathway on macrophages, thus stimulating foam cells formation, an early feature of atherosclerosis. Moreover, glycated LDL are more susceptible to oxidative modification than non-glycated LDL and glycation of LDL may alter their structure sufficiently to render them more immunogenic resulting in significant accumulation of cholesterol ester in macrophages.

Low levels of HDL constitute an independent biomarker of cardiovascular morbidity and mortality.

However, recent advances have drastically changed the classical and limited view of HDL as a carrier of "good cholesterol", and have revealed unexpected levels of complexity in the circulating HDL particle pool. HDL particles are indeed highly heterogeneous in structure with various biological activities. They are carriers of several types of lipids, proteins and evens small RNA such as microRNAs (13), and have been attributed numerous biological functions. Additionally, it has been suggested that HDL particles capacity to promote efflux of cholesterol from cells vary upon their composition in cholesterol, proteins and certain pathological conditions (14). Moreover, HDL is susceptible to structural modifications mediated by various mechanisms including oxidation, glycation, homocysteinylation or enzymatic degradation. These structural alterations may affect HDL functional and atheroprotective properties. For example, oxidants, such as hypochlorous acid, peroxynitrite, peroxyl radicals, metal ions, lipoxygenases and smoke extracts, can alter both surface and core components of HDL. The formation of lipid peroxidation derivatives, such as thiobarbituric acid reactive substances, conjugated dienes, lipid hydroperoxides and aldehydes, is associated with changes of physical properties and of apoproteins conformation. Non-enzymatic glycation of HDL generally associated with lipoxidation results in formation of irreversible complexes called advanced glycation end products that affect particles recognition by cells and induce a diminished efflux of cholesterol from cell membranes to HDL particles (15). All these modifications are accompanied with altered biological activities of HDL-associated enzymes, including paraoxonase, CETP and LCAT (16). Furthermore, homocysteine-induced modification of HDL is mediated by homocysteine-thiolactone, and can be prevented by a calcium-dependent thiolactonase/paraoxonase. Tyrosylation of HDL induces the formation of dimers and trimers of apoA-I, and alters cholesterol efflux (17). Phospholipases and proteolytic enzymes can also modify HDL lipids and apoproteins structure. High levels of nitrated apoA-I containing HDL have been reported in atherosclerotic plaques and in plasma of CVD patients (18). Circulating nitrated apoAI-HDL was markedly higher in CVD patients with low plasma HDL levels than subjects with high HDL (19). Interestingly, patients with well-functioning grafts showed significant reduction in nitrated apoAI-HDL after 12 months kidney transplantation (20). It has been also reported that nitrated apoA-I/apoA-I ratio is associated with diabetes and positively correlated with higher C-reactive protein (CRP) and TBARS levels (21). In addition, higher nitrated apoA-I/apoA-I ratio from subjects with similar apoA-I levels was significantly associated with reduced cholesterol efflux capacity from macrophages *in vitro* (21). Other modification such as carbamylation of HDL was pronounced in patients with End Stage Renal Disease (ESRD) and positively correlated with blood urea concentration. Furthermore, the activity of paraoxonase 1 (PON1)-associated HDL was decreased and negatively correlated with carbamylated HDL in ESRD patients (22). Taken together, these findings emphasize the importance of HDL and the need to identify specific sub-fractions of HDL that can provide with risk prediction either alone or in combination with other CVD biomarkers.

Although lipid and lipoprotein modification holds the key to effective secondary prevention of CVD (23, 24), there is still a controversy on whether it's all about targeting specific lipoprotein sub-fraction (s) to a particular level or a direct effect of the agent (s) used or both. Here, the questions that can be asked are: i) what is the appropriate timing of testing for lipid profile after a cardiovascular event? ii) what is the impact of targeting specific lipoprotein sub-fractions beside LDLc? iii) what constitutes a long-term lipoprotein modification strategy in CVD patients? By addressing all these very important questions, careful lipoprotein sub-fractions analysis (both quantity and quality) might prove to be one of the most successful secondary CVD preventive strategies. Furthermore, a large debate has taken place on whether a risk marker must be causally related to disease, or whether clinical utility can be advocated for a marker that might not be causal, but could indicate use of a different course of therapy or management strategy than would otherwise be considered. While traditional ALT (particles size and number) could provide some additional insights and information, it is not yet established whether these measurements would be necessary for the evaluation and stratification of the vast majority of CVD patients. Before any recommendation for routine clinical use, careful ALT including lipoprotein modification status in well-designed studies is required in order to demonstrate if such testing provides sufficient useful information for clinical trials outcome other than LDLc and non-HDLc levels.

3. CONCLUSION

We strongly believe that the present special issue provides with precise and up to date some of the pertinent information with respect to modified lipoproteins as promising biomarkers in CVD. Although assessing lipoprotein modifications along with size and particles distribution may have some clinical utility, the evidence would still be based on research laboratory quality assays, and caution is advised relative to the accuracy, reproducibility, and precision of commercially available ELISA-based assays. In fact, the exact picture of lipoprotein function is not fully understood despite careful examination of size and distribution of lipoproteins in different clinical trials setting. Sometimes it is difficult to understand the difference between LDLc levels and LDL particles and how particle size, number and

modification come into the picture. Nevertheless, benchmarks are needed against which aspect of lipoproteins analysis should be evaluated for better clinical implication in cardiovascular trials. Use of combinations of biomarkers may add prognostic value to CVD risk prediction. Exciting results with PCSK9 inhibitors (FOURIER) demonstrated that the longer your treat the patient the better the benefit. Here again, with this new concept, it would be of great interest to refine assessment of lipoproteins profile changes, especially in high CVD risk patients, not only in term of their concentrations but also gather a clear picture about the quality and function of lipoproteins that could explain the incremental benefit with the new anti-atherosclerotic therapy beyond intensive statin treatment. In evaluating the clinical potential of lipoproteins modification, the questions one certainly should ask are: i) can degree of lipoprotein modification be accurately and reproducibly measured? ii) does lipoprotein modification testing provides additional information to or improve upon existing traditional lipid profile testing?, and iii) will information on lipoprotein modification help the clinician's ability to appropriately manage the patient's cardiovascular status?

Finally, because of the current limitations for traditional ALT in clinical practice, accurate assessment of modified lipoprotein status is needed to fully gather missing information that may change our way of interpreting clinical study outcome for better practical strategies and successful patient management. Newly obtained information can be used to assess the overall risk profile, but more importantly to monitor the effectiveness of drug treatment, especially when you're trying to reduce the small dense atherogenic LDL particles. New recommendations for future research on modified lipoproteins as biomarkers may lead to a breakthrough in the diagnosis and prevention of CVD and their associated pathologies. Although there is no sufficient evidence to support immediate practical use, results from well-oriented lipid profiling with new studies will certainly answer to the question whether non-enzymatic lipoprotein modification of specific lipoprotein subspecies stands as promising biomarker for CVD or the search in this direction will need to come to an end.

4. ACKNOWLEDGMENTS

The guest editors of this special issue would like to thank all authors for their valuable contributions to the special issue and reviewers for their time and constructive feedback to authors. We would like to express our gratitude to the Editor-in-Chief of Frontiers in Bioscience, Prof. Siamak Tabibzadeh for the opportunity to edit this special issue. Our special appreciation as well to the support received by the journal production team.

5. REFERENCES

1. D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. R. Das, S. de Ferranti, J. P. Despres, H. J. Fullerton, V. J. Howard, M. D. Huffman, C. R. Isasi, M. C. Jimenez, S. E. Judd, B. M. Kissela, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. J. Magid, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolino, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, W. Rosamond, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, D. Woo, R. W. Yeh and M. B. Turner: Executive Summary: Heart Disease and Stroke Statistics–2016 Update: A Report From the American Heart Association. *Circulation*, 133(4), 447–54 (2016)
DOI: 10.1161/CIR.0000000000000366
DOI: 10.1161/CIR.0000000000000350

2. P. Shah, C. J. Glueck, N. Goldenberg, S. Min, C. Mahida, I. Schlam, M. Rothschild, A. Huda and P. Wang: Efficacy, safety, Low density lipoprotein cholesterol lowering, and calculated 10-year cardiovascular risk reduction of alirocumab and evolocumab in addition to maximal tolerated cholesterol lowering therapy: a post-commercialization study. *Lipids Health Dis*, 16(1), 19 (2017)
DOI: 10.1186/s12944-017-0416-7

3. S. Blankenberg, T. Zeller, O. Saarela, A. S. Havulinna, F. Kee, H. Tunstall-Pedoe, K. Kuulasmaa, J. Yarnell, R. B. Schnabel, P. S. Wild, T. F. Munzel, K. J. Lackner, L. Tiret, A. Evans and V. Salomaa: Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts: the MONICA, risk, genetics, archiving, and monograph (MORGAM) biomarker project. *Circulation*, 121(22), 2388–97 (2010)
DOI:10.1161/CIRCULATIONAHA.109.901413

4. S. Lewington, G. Whitlock, R. Clarke, P. Sherliker, J. Emberson, J. Halsey, N. Qizilbash, R. Peto and R. Collins: Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet*, 370(9602), 1829–39 (2007)
DOI: 10.1016/S0140-6736(07)61778-4

5. S. Oravec, Z. Krivosikova, M. Krivosik, K. Gruber, M. Gruber, A. Dukat and P. Gavornik:

Lipoprotein profile in patients who survive a stroke. *Neuro Endocrinol Lett*, 32(4), 496–501 (2011)

6. W. C. Cromwell, J. D. Otvos, M. J. Keyes, M. J. Pencina, L. Sullivan, R. S. Vasan, P. W. Wilson and R. B. D'Agostino: LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. *J Clin Lipidol*, 1(6), 583–92 (2007) DOI: 10.1016/j.jacl.2007.10.001

7. A. Kontush: HDL particle number and size as predictors of cardiovascular disease. *Front Pharmacol*, 6, 218 (2015) DOI: 10.3389/fphar.2015.00218

8. P. Libby and M. Aikawa: Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat Med*, 8(11), 1257–62 (2002) DOI: 10.1038/nm1102-1257

9. E. A. Ivanova, Y. V. Bobryshev and A. N. Orekhov: LDL electronegativity index: a potential novel index for predicting cardiovascular disease. *Vasc Health Risk Manag*, 11, 525–32 (2015)

10. A. Trpkovic, I. Resanovic, J. Stanimirovic, D. Radak, S. A. Mousa, D. Cenic-Milosevic, D. Jevremovic and E. R. Isenovic: Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit Rev Clin Lab Sci*, 52(2), 70–85 (2015) DOI: 10.3109/10408363.2014.992063

11. E. S. Lourida, A. I. Papathanasiou, J. A. Goudevenos and A. D. Tselepis: The low-density lipoprotein (LDL)-associated PAF-acetylhydrolase activity and the extent of LDL oxidation are important determinants of the autoantibody titers against oxidized LDL in patients with coronary artery disease. *Prostaglandins Leukot Essent Fatty Acids*, 75(2), 117–26 (2006) DOI: 10.1016/j.plefa.2006.03.012

12. H. Soran and P. N. Durrington: Susceptibility of LDL and its subfractions to glycation. *Curr Opin Lipidol*, 22(4), 254–61 (2011) DOI: 10.1097/MOL.0b013e328348a43f

13. M Zaiou, H EL Amri, A Bakillah. The Clinical Potential of Adipogenesis and Obesity-related MicroRNAs. *Nutr Metab Cardiovasc Dis.* 2017; under revision.

14. E. Eren, N. Yilmaz and O. Aydin: High Density Lipoprotein and it's Dysfunction. *Open Biochem J*, 6, 78–93 (2012) DOI: 10.2174/1874091X01206010078

15. B. Verges: Lipid modification in type 2 diabetes: the role of LDL and HDL. *Fundam Clin Pharmacol*, 23(6), 681–5 (2009) DOI: 10.1111/j.1472-8206.2009.00739.x

16. M. Navab, G. M. Anantharamaiah, S. T. Reddy, B. J. Van Lenten and A. M. Fogelman: HDL as a biomarker, potential therapeutic target, and therapy. *Diabetes*, 58(12), 2711–7 (2009) DOI: 10.2337/db09-0538

17. G. Ferretti, T. Bacchetti, A. Negre-Salvayre, R. Salvayre, N. Dousset and G. Curatola: Structural modifications of HDL and functional consequences. *Atherosclerosis*, 184(1), 1–7 (2006) DOI: 10.1016/j.atherosclerosis.2005.08.008

18. J. A. DiDonato, K. Aulak, Y. Huang, M. Wagner, G. Gerstenecker, C. Topbas, V. Gogonea, A. J. DiDonato, W. H. Tang, R. A. Mehl, P. L. Fox, E. F. Plow, J. D. Smith, E. A. Fisher and S. L. Hazen: Site-specific nitration of apolipoprotein A-I at tyrosine 166 is both abundant within human atherosclerotic plaque and dysfunctional. *J Biol Chem*, 289(15), 10276–92 (2014) DOI: 10.1074/jbc.M114.556506

19. A. Bakillah: Nitrated apolipoprotein A-I, a potential new cardiovascular marker, is markedly increased in low high-density lipoprotein cholesterol subjects. *Clin Chem Lab Med*, 47(1), 60–9 (2009) DOI: 10.1515/CCLM.2009.017

20. A. Bakillah, F. Tedla, I. Ayoub, D. John, A. J. Norin, M. M. Hussain and C. Brown: Plasma Nitration of High-Density and Low-Density Lipoproteins in Chronic Kidney Disease Patients Receiving Kidney Transplants. *Mediators Inflamm*, 2015, 352356 (2015) DOI: 10.1155/2015/352356

21. X. Chen, A. Bakillah, L. Zhou, X. Pan, F. Hoepfner, M. Jacob, X. C. Jiang, J. Lazar, A. Schlitt and M. M. Hussain: Nitrated apolipoprotein AI/apolipoprotein AI ratio is increased in diabetic patients with coronary artery disease. *Atherosclerosis*, 245, 12–21 (2016) DOI: 10.1016/j.atherosclerosis.2015.11.021

22. J. T. Sun, K. Yang, L. Lu, Z. B. Zhu, J. Z. Zhu, J. W. Ni, H. Han, N. Chen and R. Y. Zhang: Increased carbamylation level of HDL in end-stage renal disease: carbamylated-HDL attenuated endothelial cell function. *Am J Physiol Renal Physiol*, 310(6), F511–7 (2016)
DOI: 10.1152/ajprenal.00508.2015

23. A. M. Gotto, Jr. and J. E. Moon: Management of cardiovascular risk: the importance of meeting lipid targets. *Am J Cardiol*, 110 (1 Suppl), 3a-14a (2012)
DOI: 10.1016/j.amjcard.2012.04.002

24. I. M. Graham and A. L. Catapano: Management of Dyslipidemias in Europe and the USA: Same Evidence, Different Conclusions? Can We Find Common Ground? *Curr Cardiol Rep*, 19(6), 49 (2017)
DOI: 10.1007/s11886-017-0857-7

Key Words: Biomarkers, Cardiovascular diseases, ELISA, Lipoproteins, Lipoprotein modification, Review

Send correspondence to: Ahmed Bakillah, SUNY Downstate Medical Center, Department of Medicine, 450 Clarkson Ave, Brooklyn, NY-11203, USA, Tel: 718-270-2216, Fax: 718-270-3899, E-mail: Ahmed.Bakillah@Downstate.edu