Original Research Article

Serum Level of Lipoprotein-Associated Phospholipase A2 and Coronary Artery Disease

Abstract

Background: Cardiovascular diseases (CVDs) are on the rise owing to excessive fat consumption or hereditary factors. From infancy through old age, it is the leading cause of disease and mortality. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme mostly excreted by macrophages and neutrophils from atherosclerotic plaque, which subsequently circulates in the circulation. We aimed to evaluate the relation between plasma level of LP-PLA2 and severity of coronary artery disease (CAD).

Methods: This retrospective randomized case control study was conducted on 60 cases aged ≥ 18 years, presented by any form of CAD than ten healthy adults to determine the relation between plasma level of LP-PLA2 and severity of CAD. All cases were subjected to the following: Full history taking such as Socio-demographic study including age, sex, occupation, complain, history of illness: Including family history of CAD, history of smoking, hypertension and diabetes mellitus (DM).

Results: Significantly elevated LDL-C, LP-PLA2, and decreased HDL-C levels were also observed in patients with significant CAD. The HDL-C levels of individuals with CAD were significantly lower than those of healthy individuals. HDL-C levels in Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) were 78.5, 38.95, 36.85, 32 units?, respectively; there was highly significance relation between both group regarding HDL-C Level with (p-value=0.003). Plasma level of Lp-PLA2 was gradually elevated in parallel with the increased severity of CAD. There was highly significant difference among study groups regarding plasma level of Lp-PLA2. Lp-PLA2 plasma level in Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) were 87.9, 216.95, 272.15, 379.95 units?,

respectively; (p-value=0.001) show gradually significant elevation. Lp-PLA2 level remained independently associated with coronary artery stenosis after adjustment for age, gender, smoking, diabetes, hypertension, LDL-C and HDL-C.

Conclusions: An increased plasma level of Lp-PLA2 is related with an unstable phenotype of coronary atherosclerotic plaque and a greater degree of coronary artery stenosis in individuals with coronary artery disease (CAD). Dynamic Lp-PLA2 monitoring may be predictive of cardiovascular disease risk. In recent years, there has been great interest in the capacity of Lp-PLA2 to predict and evaluate the prognosis of CVDs. In addition, Lp-PLA2 has been identified as an independent risk factor for the development and progression of coronary heart disease..

Keywords: Serum Level of Lipoprotein-Associated Phospholipase A2**,** Coronary Artery Disease

Introduction:

Cardiovascular diseases (CVDs) are increasing as a result of excessive fat intake or genetics. From infancy through old age, it is the leading cause of disease and mortality. Despite the availability of conventional risk prediction techniques for the presence of important CVDs risk factors in sick populations, there are no reliable and accurate biomarkers for CVDs. It not only delayed clinical diagnosis, but also dramatically increased risk and caused unexpected deaths. To accelerate disease prevention and morbidity reduction, it is necessary to promptly identify and manage risk factors. [1] Several risk scores to predict CVDs risk have been developed. This article provides a summary of several biomarkers for predicting CVDs risk by including both old risk factors to be used as diagnostic markers and recently found diagnostic and therapeutic markers. [2]

Ravi Kant Upadhyay analysed conventional biomarkers like lipid profile, glucose, and hormone level, as well as physiological biomarkers based on measurement of levels of essential biomolecules like serum ferritin, triglyceride to high density lipoproteins ratio (HDLp), lipoprotein-cholesterol ratio, lipid-lipoprotein ratio, low density lipoprotein (LDL) cholesterol level, and HDLp and apolipoprotein levels. In alone or in combination, a number of these biomarkers may play a crucial role in the prediction of risks, their types, and the degree of morbidity.[2]

Endothelial dysfunction is the major cause of the start and progression of atherosclerosis. Therefore, after a period of exposure to classic risk factors such as obesity, smoking, hypertension (HTN), dyslipidemia, and diabetes, endothelial cells become dysfunctional, the natural barrier between endothelia is disrupted, and lipids begin to accumulate in sub-endothelial spaces. [3]

The hydrolysis of oxLDL is a significant source of inflammatory cytokines, reactive oxygen species, and chemotactic factors produced by macrophages. In the aftermath, more leukocytes

penetrate and accumulate, lipid oxidation becomes more intense, endothelial cells degrade, and vascular inflammation increases. Multiple vicious cycles lead to the progressive development of atherosclerotic plaque, which is characterised by a necrotic lipid core, inflammatory cells, and a fibrous cap, and which increases the risk of CVDs disease events.[4] Numerous metrics have been developed to assess the stability of atherosclerotic plaque, which is essential for the development of cardiovascular diseases. A plaque with a thinner fibrous top and a greater proportion of necrotic lipid core is more prone to rupture than plaques with other morphologies. Therefore, detecting a prone rupture plaque would be advantageous and helps to avoid CVDs events. [4] In a limited number of studies, intravascular ultrasonography and carotid magnetic resonance imaging have been used efficiently to assess the composition of atherosclerotic plaques. However, the invasiveness, price, and complexity of these procedures prevent their broad use. Multiple studies demonstrate that the activity of Lp-PLA2 in plaques that are prone to rupture is much greater than in stable plaques with a tiny lipid core and a thick fibrous cap. [5, 6]

Lipoprotein-associated phospholipase A2 (Lp-PLA2) activity or mass is highly connected to plaque stability, and Lp-PLA2 may be helpful and trustworthy for detecting fragile plaques and estimating future CVDs risk. [7, 8] Lp-PLA2 is an enzyme mostly excreted by macrophages and neutrophils from atherosclerotic plaque, which subsequently circulates in the circulation.[9, 10]

70 % to 80 % of Lp-PLA2 circulates bound to LDL, whereas the remaining is bound to HDL, lipoprotein (a) [Lp(a), and extremely low-density lipoproteins (VLDL)[11, 12], and once in the arterial wall facilitates hydrolysis of phospholipids. [13, 14] Lp-PLA2 was first identified in vitro as platelet activating factor acetyl hydrolase ,an enzyme that degrades the inflammatory mediator PAF.[15] So, LP-PLA2 plays a complex and adverse role in atherosclerosis. [10]

We conducted an observational clinical study to examine if a higher plasma level of LP-PLA2 is independently associated with the severity of coronary artery disease (CAD) and whether LP-PLA2 might be used to predict CAD risk in the future. We sought to determine the relationship between plasma levels of LP-PLA2 and CAD severity. Novelty?

Patients and Methods:

This observational clinical study was conducted on 60 patients to examine if a higher plasma level of LP-PLA2 is independently associated with the severity of CAD.A documented informed consent was received from the patient or the patient's family. The study was conducted with the approval of the Ethical Committee of Tanta University Hospitals beginning in January 2016 and continuing until the present (January 2018).

Coronary angiography was carried out to count the number of coronary artery stenosis, either as a single vessel or multiple vessel stenosis.(≥ 50%).

Exclusion criteria were type 1 DM, in control group people who >50%stenosis detected by coronary angiography, unable to written consent.

All patients were submitted to the following: A complete history that includes socio-demographic details such as age, gender, occupation, and complaints. Includes coronary artery disease in the family, smoking, hypertension, and diabetes.

BP measurement is the focal point of the clinical evaluation. Clinical manifestation, ECG results, cardiac biomarkers, and coronary angiography comprise a comprehensive clinical evaluation. Laboratory experiments: Standard investigations, glucose level when fasting, Total cholesterol, Triglyceride, LDL-C, HDL-C, and glycosylated haemoglobin.

Specific investigations: Eight millilitres (8 ml) of blood were collected from each patient who had fasted for eight to twelve hours under strict aseptic conditions. 2.0 mL were collected into sterile tri potassium ethylene diamine (K3 EDTA) vacutainers tubes; the remaining blood was placed in 2 sterile vacutainers with a clot activator and allowed to clot

for 30 minutes; serum was then separated by centrifugation at 1000 x g for 15 minutes; one aliquot for immediate assay of routine lab investigations and the remaining part of sera was aliquoted. Before analysis, frozen samples were simply defrosted and brought to room temperature. Homolyzed specimens were discarded, and repetitive cycles of freezing and thawing were avoided. Method for determination of Lp-PLA2?

Statistical analysis:

SPSS v27 (IBM, Armonk, NY, USA) was used for the statistical analysis. Using histograms and the Shapiro-Wilks test, the normality of the data distribution was determined. Parametric quantitative data was summarised as mean SD and examined using an unpaired student t-test. The Mann Whitney-test was used to assess the non-normal distribution of the quantitative data, and the median and interquartile range (IQR) were used to summarise the distribution. Quantitative data were given as means and standard deviations, while qualitative variables were shown as frequencies and percentages and examined using the Chi-squared test or Fisher's exact test, as applicable. The odds of an event occurring are computed as the ratio of the likelihood of a property's presence to its absence; this is simply the number of times the property has been present divided by the number of times it has been absent. The odds ratio is computed by dividing the patient group's odds by the control group's odds. A two-tailed P value of less than or equal to 0.05 was considered statistically significant.

Results:

This is a retrospective randomized case-control study that was conducted on sixty participants who were angiographically diagnosed as having CAD and ten who will rule out of CAD to evaluate the relation between plasma level of LP-PLA2 and severity of CAD.

Subjects were classified into the following groups.

Control group (n=10): who are without coronary artery stenosis and patient groups (n=60): divided according to severity of the disease

According to clinical presentation:

- Group A (n=20): CAD patients with stable angina (SA)
- Group B (n=20): CAD patients with unstable angina (unSA)
- Group C (n=20): CAD patients with acute myocardial infarction (MI)

Single (n=17) or multiple (n=34) coronary artery stenosis, according to the number of coronary artery stenosis.

Mean ages in the control (n = 10), SA (n = 20), UA (n = 20) and AMI (n = 20) groups were 57 ± 11.6, 61.7 ± 9.84, 63.1 ± 10.47 and 64.6 ± 10.24 years, respectively with no statistically significant difference. This Statistical comparison between control group (N=10) and CAD group (N=60) regarding Age shows no statistically significant difference and p-value=0.094. There was Statistically significant relation between Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) regarding gender. Patients with significant CAD were more likely to be Male with frequencies of 15 (75%), 17 (85%) and 17 (85%) in SA, UA and AMI group, respectively compared to 4 (40%) in control group.

Compared to controls, those with verified CAD had higher conventional risk variables. Patients with severe CAD were more likely to have a history of congestive heart failure or myocardial infarction, smoking, and HTN. There was a significant difference between Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) regarding HTN; shows statistically significant difference with (p-value=0.006). There was a significant relation between Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) regarding history of AMI, it shows statistically significant difference with (p-value=0.029). Patients with significant CAD were more likely to have history of AMI with frequencies of 7 cases, 9 cases and 11 cases in SA, UA and AMI group, respectively. **Table 1**

Table 1: Baseline characteristics of the studied groups

Group	Control	SA	UA	AMI	P

	(n=10)	(n=20)	(n=20)	(n=20)	
Age (years)	57 ± 11.6	61.7 ± 9.84	63.1 ± 10.47	64.6 ± 10.24	0.094
Male (%)	4 (40)	15 (75)	17 (85)	17 (85)	**0.032***
Female (%)	6 (60)	5 (25)	3 (15)	3 (15)	
Family history (%)	1 (10%)	3 (15%)	4 (20%)	6 (30%)	0.534
History of AMI (%)	0 (0%)	7 (35%)	9 (45%)	11 (55%)	**0.029***
History of CHF (%)	1 (10%)	10 (50%)	11 (55%)	13 (65%)	**0.038***
Smoking (%)	1 (10%)	9 (45%)	10 (50%)	13 (65%)	**0.042***
Hypertension (%)	1 (10%)	12 (60%)	13(65%)	15(75%)	**0.006***
Type 2Diabetes mellitus (%)	3 (30%)	6 (30%)	4 (20%)	7 (35%)	0.764

Patients with significant CAD also had significant increase of LDL -C and LP-PLA2 level and significant decrease of HDL C level compared to control. HDL-C were significantly lower in participants with CAD as compared to the controls. HDL-C levels in Control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) were 78.5, 38.95, 36.85, 32, respectively; there was highly significance relation between both group regarding HDL-C Level with (p-value=0.003). **Table 2**

Table (2): Lipid profile total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, blood glucose level and Lp-PLA2 in studied groups:

Group	Control (n=10)	SA (n=20)	UA (n=20)	AMI (n=20)	P
FBG (mg/dl)	109.9 ± 6.6	112.5 ± 8.4	108.6 ± 7.5	115.3 ± 9.7	0.113
HbA1c (%)	4.75 ± 1.59	5.74 ± 0.47	4.91 ± 0.36	5.66 ± 0.49	0.218

TC (mg/dl)	173.6 ± 7.51	290.5 ± 35.67	287.1 ± 35.28	280.2 ±33.19	0.409
TG (mg/dl)	224.3 ± 15.51	231.55±51.4	257.9 ± 31.35	245.2 ±36.95	0.197
LDL-C (mg/dl)	89.7 ± 4.34	200.45±6.31	215.25 ± 9.36	225.62 ± 20.06	0.004*
HDL-C (mg/dl)	78.5±5.23	38.95±2.031	36.85 ± 1.98	32 ±2.02	0.003*
Lp-PLA2 (ng/ml)	87.9±4.53	216.95±13	272.15 ± 11.34	379.95 ±16.96	0.001*

The plasma concentration of Lp-PLA2 rose gradually alongside the progression of CAD severity. There were highly significant differences in plasma Lp-PLA2 levels between study groups.Lp-PLA2 plasma level in control group (N=10) and CAD groups (SA=20, UA=20 and AMI=20) were 87.9, 216.95, 272.15, 379.95, REPEATS! respectively; (p-value=0.001) show gradually significant elevation**. Table 3**

Table (3): Serum Lp-PLA2 level by ng/ml in studied groups – NOT CLEAR!

Group	**Control (n=10)**	**SA (n=20)**	**UA (n=20)**	**AMI (n=20)**	**P**	**OR (95% CI)**
Lp-PLA2 (ng/ml)	**87.9 ± 4.53**	**216.95 ± 13**	**272.15 ± 11.34**	**379.95 ±16.96**	**0.001**	**3.931 (2.591 – 8.524)**
Control & SA	**Control & UA**	**Control & AMI**	**SA & UA**	**SA & AMI**	**UA & AMI**	
0.001	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	

Lp-PLA2 level remained independently associated with the number of coronary artery stenosis after adjustment for age, gender, smoking, DM, HTN, LDL-C and HDL-C. **Table 4**

Table (4)**: Relationship between Lp-PLA2 level and the number of coronary artery stenosis:**

	Control (n = 10)	Single-vessel stenosis (n = 17)	Multiple-vessels stenosis (n = 43)	P-value	OR (95% CI)
Lp-PLA2 ng/ml	**87.9±4.53**	**327.53±12**	**363±14**	**< 0.05**	**1.023 (1.013-1.067)**
	Control & Single	**Control & Multiple**	**Single & Multiple**		
p-value	**0.001**	**0.001**	**0.008**		

Discussion

Pathologically, Lp-PLA2 aggravates inflammation and oxidation by degrading oxidized-LDL into two potent pro-atherosclerotic and prothrombotic intermediates, lysophosphotidylcholine (Lyso-PC) and oxidised non-esterified fatty acids (oxNEFAs), causing endothelial dysfunction, foam cell formation, necrotic lipid-core expansion, and fibrous. [16]

In addition, clinical and basic research demonstrated that decreasing LP-LPA2 slowed the progression of atherosclerosis and reduced cardiovascular events. In an animal model of hyperlipidaemia and hyperglycaemia, for instance, Lp-PLA2 inhibitor reduced macrophage accumulation, decreased necrotic lipid-core volume, and thickened fibrous cap of coronary atherosclerotic plaque relative to placebo treatment.. [17, 18]

Therefore, it is plausible to hypothesise that higher plasma levels of PL-PLA2 may contribute to the development of coronary artery stenosis and accelerate the rupture of atherosclerotic plaque.

Earlier clinical epidemiological studies demonstrated that a higher plasma level of PL-PLA2 was associated with an increased risk of CV events such as myocardial infarction and ischemic stroke, and that LP-LPA2 inhibitors could significantly reduce the incidence of cardiovascular events compared to placebo treatment. [19-21]

Data from basic experiments further supported this clinical finding. [17, 18, 22, 23] Therefore, LP-LPA2 has been incorporated into CVDs risk assessment algorithm. [24]

Two significant randomised, controlled, and prospective clinical studies indicated that the selective LP-LPA2 antagonist did not improve clinical outcomes in persons with stable or unstable coronary artery disease, despite encouraging early research findings. Despite optimistic results from early research, two major randomised, controlled, and prospective clinical trials found that the selective LP-LPA2 antagonist did not enhance clinical outcomes in individuals with SA or unSA CAD. [25, 26]

In our investigation, Several risk scores have been established to predict the risk of CVDs. This article presents an overview of numerous biomarkers for predicting the risk of cardiovascular diseases, covering both traditional risk variables to be utilised as diagnostic markers and newly discovered diagnostic and therapeutic markers. LDL-C, and HDL-C, the LP-LPA2 level remained independently associated with the severity of CAD.(OR) 3.931. (AMI vs control group 95% CI 2.591-8.524 $p < 0.001$).

This may provide credence to the theory that elevated plasma levels of LP-LPA2 indicate a more unstable coronary artery plaque phenotype. Therefore, it is probable that when LP-LPA2 levels increase, endothelial dysfunction and atherosclerosis become more severe.

This is in agreement with ***Caslake et al.,*** [27] who demonstrated that LP-LPA2 levels were higher in 94 patients with CAD than in 54 controls. The connection remained even after controlling for LDL and HDL-cholesterol, smoking, and systolic blood pressure.. [28]

This finding was also consistent with previous reports. Data from ***Kolodgie et al.,*** [29] shown that LP-LPA2 staining was more intense in human coronary atheroma with a propensity for rupture than in plaques with a reasonably stable composition.

LP-LPA2 concentrations were considerably greater in cases than in controls, as corroborated by ***Khuseyinova et al.,*** [30],.'s research of patients with angiographic evidence of CAD and

age- and gender-matched blood donors. After adjusting for conventional CV risk variables and statin use, the adjusted OR was 2.04 (1.19-3.48).

Brilakis et al., [31] performed On 504 individuals receiving clinically essential coronary angiography, further research was undertaken. This connection persisted after adjusting for clinical, metabolic, and CRP factors..[32]

Based on angiographic data, the findings of our study revealed a link between LP-LPA2 concentration and the number of coronary artery stenosis. Participants were divided into control, single, and multiple vascular stenosis groups.LP-LPA2 levels, in the control (n=10), single (n=17), multiple (n=43) groups. Plasma levels of LP-LPA2 were significantly higher in persons with single and multiple vascular stenosis (n=17 and n=43, respectively) compared to those without severe CAD (n=17 and n=43, respectively). Early coronary atherosclerosis in humans was characterised by LP-LPA2 synthesis, and endothelial dysfunction and atherogenesis were associated with local coronary production of lyso-PC, the active product of LP-LPA2., according to studies by Lavi and colleagues [33, 34].

The accumulation of evidence from basic and clinical investigations may be adequate to demonstrate the positive association between higher LP-LPA2 concentrations and endothelial dysfunction. Lyso-PC and oxNEFAs, in addition to endothelium-associated pathways, play important and complicated roles in the development of atherosclerotic plaques. Lyso-PC and oxNEFAs are able to upregulate the expression of intracellular adhesion molecules (ICAM), hence boosting inflammatory cell infiltration and accumulation, encouraging foam cell formation, and extending necrotic lipid core. Thus, LP-LPA2 and its product begin and accelerate the formation of atherosclerotic plaques simultaneously.

LDL-C, HDL-C, and LP-LPA2 levels remained independently associated with the occurrence of coronary artery stenosis, with OR of 1.013 (multiple-vessel stenosis group vs. control group, p 0.05),95% [CI]: 1.023-1.067, P 0.05)., 95% (CI) 1.023-1.067, P < 0.05)

As regards serum lipids, our results showed that patients with severe CAD had significantly higher LDL-C and significantly lower HDL-C compared to the control group, and these changes were significantly associated with the severity of CAD, suggesting that dyslipidaemia may be associated with the severity of CAD. Nonetheless, after adjusting for these lipid profiles, the LP-LPA2 level remained independently linked with the clinical severity of CAD with OR= 3.931.

Similarly with previous results, **Daida et al., [35]** found a linear relationship between the incidence of cardiovascular disease and LDL-C in high-risk hypercholesterolemia individuals.

Chieng et al., [36] In individuals with early CAD, increased lipoprotein (a) and LDL-C were significant and independent indicators of CAD severity. Therefore, we hypothesised that an increasing LP-PLA2 level may serve as a predictor of future cardiovascular events such as acute coronary syndrome.

Our study had several limitations, The sample size of this study was relatively small, and therefore, the results should be interpreted cautiously.

Conclusions:

A higher degree of coronary artery stenosis and an unstable phenotype of coronary atherosclerotic plaque are linked to increased plasma levels of Lp-PLA2 in people with coronary artery disease. Keeping an eye on fluctuating Lp-PLA2 levels could be an indicator of future CV problems. The use of Lp- PLA2 for the diagnosis and prognosis of cardiovascular disease has gained popularity in recent years. Additionally, Lp-PLA2 is an independent risk factor for the onset and progression of coronary heart disease. It is necessary to establish a reference interval for Lp-PLA2 levels for use in the clinical evaluation of patients with CVDs. By accounting for the effects of age and gender on Lp-PLA2 levels, our

results not only set a new benchmark for therapeutic practise, but they also pave the way for future research in this field.

References:

1. Bamba V. Update on screening, etiology, and treatment of dyslipidemia in children. J Clin Endocrinol Metab.99:3093-102. 2014

2. Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. J Lipids.2015:971453. 2015

3. Fordjour PA, Wang Y, Shi Y, Agyemang K, Akinyi M, Zhang Q, et al. Possible mechanisms of C-reactive protein mediated acute myocardial infarction. Eur J Pharmacol.760:72-80. 2015

4. Tuzcu EM, Kapadia SR, Tutar E, Ziada KM, Hobbs RE, McCarthy PM, et al. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: evidence from intravascular ultrasound. Circulation.103:2705-10. 2001

5. Herrmann J, Mannheim D, Wohlert C, Versari D, Meyer FB, McConnell JP, et al. Expression of lipoprotein-associated phospholipase A(2) in carotid artery plaques predicts long-term cardiac outcome. Eur Heart J.30:2930-8. 2009

6. Hetterich H, Jaber A, Gehring M, Curta A, Bamberg F, Filipovic N, et al. Coronary computed tomography angiography based assessment of endothelial shear stress and its association with atherosclerotic plaque distribution in-vivo. PLoS One.10:e0115408. 2015

7. Mannheim D, Herrmann J, Versari D, Gössl M, Meyer FB, McConnell JP, et al. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. Stroke.39:1448-55. 2008

8. Tehrani DM, Wong ND. Cardiovascular Disease Risk Assessment: Review of Established and Newer Modalities. Curr Treat Options Cardiovasc Med.17:57. 2015

9. Macphee CH, Nelson J, Zalewski A. Role of lipoprotein-associated phospholipase A2 in atherosclerosis and its potential as a therapeutic target. Curr Opin Pharmacol.6:154-61. 2006

10. Cai A, Li G, Chen J, Li X, Li L, Zhou Y. Increased serum level of Lp-PLA2 is independently associated with the severity of coronary artery diseases: a cross-sectional study of Chinese population. BMC Cardiovasc Disord.15:14. 2015

11. Tellis CC, Tselepis AD. The role of lipoprotein-associated phospholipase A2 in atherosclerosis may depend on its lipoprotein carrier in plasma. Biochim Biophys Acta.1791:327-38. 2009

12. Tselepis AF, Rizzo M, Goudevenos IA. Therapeutic modulation of lipoprotein-associated phospholipase A2 (Lp-PLA2). Curr Pharm Des.17:3656-61. 2011

13. Epps KC, Wilensky RL. Lp-PLA$_2$- a novel risk factor for high-risk coronary and carotid artery disease. J Intern Med.269:94-106. 2011

14. Tellis CC, Tselepis AD. Pathophysiological role and clinical significance of lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) bound to LDL and HDL. Curr Pharm Des.20:6256-69. 2014

15. Rosenson RS, Stafforini DM. Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A2. J Lipid Res.53:1767-82. 2012

16. Yang L, Liu Y, Wang S, Liu T, Cong H. Association between Lp-PLA2 and coronary heart disease in Chinese patients. J Int Med Res.45:159-69. 2017

17. Wilensky RL, Shi Y, Mohler ER, 3rd, Hamamdzic D, Burgert ME, Li J, et al. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. Nat Med.14:1059-66. 2008

18. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev.111:6130-85. 2011

19. Daniels LB, Laughlin GA, Sarno MJ, Bettencourt R, Wolfert RL, Barrett-Connor E. Lipoprotein-associated phospholipase A2 is an independent predictor of incident coronary heart disease in an apparently healthy older population: the Rancho Bernardo Study. J Am Coll Cardiol.51:913-9. 2008

20. Mohler ER, 3rd, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, et al. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. J Am Coll Cardiol.51:1632-41. 2008

21. Berger JS, Ballantyne CM, Davidson MH, Johnson JL, Tarka EA, Lawrence D, et al. Peripheral artery disease, biomarkers, and darapladib. Am Heart J.161:972-8. 2011

22. Serruys PW, García-García HM, Buszman P, Erne P, Verheye S, Aschermann M, et al. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. Circulation.118:1172-82. 2008

23. Hu MM, Zhang J, Wang WY, Wu WY, Ma YL, Chen WH, et al. The inhibition of lipoprotein-associated phospholipase A2 exerts beneficial effects against atherosclerosis in LDLR-deficient mice. Acta Pharmacol Sin.32:1253-8. 2011

24. Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, Brown AS, et al. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. J Clin Lipidol.5:338-67. 2011

25. White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, et al. Darapladib for preventing ischemic events in stable coronary heart disease. N Engl J Med.370:1702-11. 2014

26. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, et al. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. Jama.312:1006-15. 2014

27. Caslake MJ, Packard CJ. Lipoprotein-associated phospholipase A2 (platelet-activating factor acetylhydrolase) and cardiovascular disease. Curr Opin Lipidol.14:347-52. 2003

28. Ulrich C, Trojanowicz B, Fiedler R, Kohler F, Wolf AF, Seibert E, et al. Differential Expression of Lipoprotein-Associated Phospholipase A2 in Monocyte Subsets: Impact of Uremia and Atherosclerosis. Nephron.135:231-41. 2017

29. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol.26:2523-9. 2006

30. Khuseyinova N, Imhof A, Rothenbacher D, Trischler G, Kuelb S, Scharnagl H, et al. Association between Lp-PLA2 and coronary artery disease: focus on its relationship with lipoproteins and markers of inflammation and hemostasis. Atherosclerosis.182:181-8. 2005

31. Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. Eur Heart J.26:137-44. 2005

32. Jiang R, Chen S, Shen Y, Wu J, Chen S, Wang A, et al. Higher Levels of Lipoprotein Associated Phospholipase A2 is associated with Increased Prevalence of Cognitive Impairment: the APAC Study. Sci Rep.6:33073. 2016

33. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, et al. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. Circulation.115:2715-21. 2007

34. Ahmadi A, Stone GW, Leipsic J, Serruys PW, Shaw L, Hecht H, et al. Association of Coronary Stenosis and Plaque Morphology With Fractional Flow Reserve and Outcomes. JAMA Cardiol.1:350-7. 2016

35. Daida H, Teramoto T, Kitagawa Y, Matsushita Y, Sugihara M. The relationship between low-density lipoprotein cholesterol levels and the incidence of cardiovascular disease in high-risk patients treated with pravastatin: main results of the APPROACH-J study. Int Heart J.55:39-47. 2014

36. Chieng D, Pang J, Ellis KL, Hillis GS, Watts GF, Schultz CJ. Elevated lipoprotein(a) and low-density lipoprotein cholesterol as predictors of the severity and complexity of angiographic lesions in patients with premature coronary artery disease. J Clin Lipidol.12:1019-26. 2018