Physiological Research Pre-Press Article

The importance of age and statin therapy in the interpretation of Lp-PLA$_2$ in ACS patients, and relation to CRP

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Short title: **Lp-PLA$_2$ vs. statin therapy in acute coronary syndrome**

Summary

C-reactive protein (CRP) is a marker of arterial inflammation while lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is related to plaque instability. The aim of this study was to evaluate the correlation between the risk of unstable plaque presenting as acute coronary syndrome (ACS) and Lp-PLA_2, and to assess the influence of statins on interpretation of Lp-PLA_2. A total of 362 consecutive patients presenting to the emergency department (ED) with acute chest pain suggestive of ACS were evaluated by cardiologists as STEMI, NSTEMI, or unstable angina, and non-ACS. Serum biomarkers measured on admission: troponin I, C-reactive protein (Abbott), and Lp-PLA_2 (DiaDexus). Four groups were defined according to the final diagnosis and history of statin medication: ACS/statin-; ACS/statin+; non-ACS/statin-; non-ACS/statin+. Lp-PLA_2 was highest in ACS/statin- group; statins decreased Lp-PLA_2 both in ACS and non-ACS of about 20%. Lp-PLA_2 was higher in ACS patients in comparison with non-ACS patients group without respect to statin therapy ($p<0,001$). Lp-PLA_2 predicted worse outcome (in terms of acute coronary syndrome) effectively in patients up to 62 years; limited prediction was found in older patients. C-reactive protein (CRP) failed to discriminate four groups of patients. Statin therapy and age should be taken into consideration while interpreting Lp-PLA_2 concentrations and lower cut-off values should be used for statin-treated persons.

Key words: Plaque, Atherosclerotic; C-Reactive Protein; Phospholipase A2; Biological Markers; Acute Coronary Syndrome

Introduction

Acute coronary syndrome (ACS) is a common complication of atherosclerotic lesions and plaque instability. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an independent marker of cardiovascular risk in relation to vulnerable plaque. Hence, increased concentrations of Lp-PLA_2 can indicate an increased risk of ACS. Statin therapy decreases Lp-PLA_2 concentrations (Chu et al. 2012, Tousoulis et al., 2012), but common algorithms use Lp-PLA_2 cut-off values for increased risk without respect to statin therapy (Davidson et al. 2008, Braun and Davidson 2010, Mohler et al. 2008, Wilensky and MacPhee 2009). CRP is a widely used marker of cardiovascular risk; however, its role in ACS, namely in polymorbid patients and emergency clinical settings, is questionable. The purpose of our study was to compare Lp-PLA_2 and CRP in two groups of patients in cardiology emergency – with and without ACS. Also, we tried to evaluate the influence of statin therapy on Lp-PLA_2 concentrations in ACS patients in comparison to patients with other heart problems. We hypothesized that the highest concentrations of Lp-PLA_2 will be measured in ACS patients without statins as a pathogenic factor for plaque instability and increased cardiovascular risk.

Methods

Lp-PLA_2 (PLAC Test ELISA kit, DiaDexus, Inc., South San Francisco, California, USA, http://diadexus.com) and CRP (Abbott Laboratories, Abbott Park, Illinois, U.S.A, www.abbott.com) were measured in 362 consecutive patients immediately after admission in the emergency department, mostly due to acute chest pain. Clinical status was evaluated by cardiologists by means of common procedures including clinical history, physical examination, ECG evaluation, and troponin I (Abbott Architect analyser, cut-off 0.03 μg/l) for the detection of myocardial injury at admission. Results of Lp-PLA_2 and CRP were blinded for emergency physician. Final diagnosis of ACS (STEMI, NSTEMI, unstable angina) or

non-ACS was made after discharge of the patients according to current guidelines. The group of non-ACS patients comprised arrhythmias (N=52), heart failure (N=39), cardiomyopathy (N=8), pulmonary embolism (N=7), hypertonic crisis (N=7), and other (N=80, a very broad spectrum of differential diagnoses of acute chest pain: cholangitis, pneumonia, vertebrogenic algic syndrome, aneurysm etc.). Statins used before admission were evaluated from the hospital information system. Retrospective analysis of the clinical data was made by one of the authors (JF). Four groups of patients were defined: 1) patients with ACS (both ST elevation myocardial infarction, STEMI, and non-ST elevation myocardial infarction, NSTEMI, unstable angina), without statin therapy, 2) ACS with statin therapy, 3) patients without ACS, without statins, 4) patients without ACS, but on statin therapy. The Kruskal-Wallis, chi-square, Mann-Whitney tests, ROC analysis, and logistic regression analysis were used for statistical evaluation with the aid of R package version 2.3.1 (R Development Core Team 2011, Harrell 2011) and MedCalc, version 13.2.0.0 (MedCalc Statistical Software version 13.2.0 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014). The study was performed in accordance with the Declaration of Helsinki and approved by IKEM Ethical Committee (REC number: MEK – 2442/10/A 11-01-02).

Results

The entire set of patients comprised 248 men (median age 65 years) and 114 women (median age 71 years). In 10 of the 362 patients there was no information on statin therapy before admission and these patients were not analysed; therefore, 352 patients were evaluated. There was no difference among ACS and non-ACS patients for age, sex, and CRP. Median age in patients on statin therapy was 68 years (interquartile range 61–76 years), patients without statins were younger (median 64, range 52–74 years, $p<0,002$). Lp-PLA_2 was significantly

higher in patients with ACS in comparison to patients without ACS, irrespective of statin therapy ($p<0.0001$).

Medians (25–75th percentile) of measured variables for 4 groups are given in Table 1; the difference in groups (p) was assessed by the non-parametric Kruskal-Wallis test. All data for Lp-PLA_2 and CRP are in Figure 1 and Figure 2. Lp-PLA2 concentration in patients without statin treatment is significantly higher in ACS than in nonACS patients. Similarly, in patients with statin treatment, the concentration of Lp-PLA2 is significantly higher in ACS than in nonACS patients.

Table 1

Figure 1

Figure 2

A restricted cubic spline surface in two variable logistic regression analysis (age, Lp-PLA_2) is displayed in Figure 3 for men and women separately (a natural logarithm of odds for ACS on vertical axis is used as a measure of clinical outcome). The surface characterises the interaction among probability of ACS, age, and Lp-PLA_2. The ability of Lp-PLA_2 to identify the patients with increased risk of ACS is more pronounced in the lower tercile of age (up to 62 years). The left panel describes the situation in men with generally higher risk of ACS, while the right panel describes the situation in women. Trends are similar in men and women; after initial increase of ACS probability with increasing age and higher concentrations of Lp-PLA_2 there is a decrease of ACS probability and slight increase thereafter. Table 2 describes results of logistic regression analysis for clinical outcome as a dependent variable (ACS or non-ACS), when age, sex, and Lp-PLA_2 were used as independent variables. The significant

coefficient was for Lp-PLA_2 only, the highest area under the curve (AUC) and the best reclassification was found for model consisting of age, sex, and Lp-PLA_2 in patients under 62 years.

Figure 3

Table 2

A restricted cubic spline surface in two variable logistic regression analysis (age, Lp-PLA_2) is displayed in Figure 4 for patients treated with statins and those without statin therapy (natural logarithm of odds for ACS on vertical axis). Without statins (left panel), there is a significant contribution of Lp-PLA_2 to the recognition of possibly unstable plaque and the risk of resultant ACS in patients in lower tercile of age (below 62 years) and older patients (above 73 years). On the other hand, there is no evident ridge in the central surface area of the right panel, presumably as a result of statin therapy.

Figure 4

Discussion

Lp-PLA_2 is directly involved in atherogenesis. Activity of metalloproteinases is increased due to production of inflammatory mediators, oxidative stress with lysophosphatidylcholine and oxidized fatty acids, and dyslipoproteinemia. As a result, thin fibrous cap and unstable plaque can be the direct cause of acute coronary syndrome (Charniot et al. 2013, Holstalbrechtsen et al. 2013, Liu et al. 2014, Lerman and McConnell 2008, Toth et al. 2010, Vickers et al. 2009) or re-stenosis after stent placement (Zheng et al. 2014). Histopathology studies have proven an increased amount of Lp-PLA_2 in unstable plaques with thin fibrous cap and large lipid

core, which are more vulnerable. It seems that Lp-PLA_2 is more related to plaque quality than extent. Reference values (Mayo Clinic Reference Value Donors Program) are higher in men (266 μg/l±48 μg/l) than in women (227 μg/l±64 μg/l). Lp-PLA_2 is associated with the progression of atherosclerosis more in men than in women (Liu et al. 2014). It is recommended to use Lp-PLA_2 for risk stratification in middle-aged persons together with risk scores (Framingham, SCORE). The cut-off value according to a consensus expert panel is 200 μg/l (Davidson et al. 2008). Intraindividual biological variability of Lp-PLA_2 is 15%, interindividual 22%, critical difference 47% (Khuseyinova and Koenig 2007).

Lp-PLA_2 is a candidate marker of cardiovascular risk with many properties of an suitable biomarker (Wang 2011), in relation to pathophysiology of atherogenesis and the development of unstable plaque. Lp-PLA_2 was proved as an efficient marker in many studies and meta-analyses and has been introduced into guidelines for optimal care of patients with moderate to high cardiovascular risk. A meta-analysis of 32 studies demonstrated a linear relationship between Lp-PLA_2 concentration (or activity) and vascular risk in primary and secondary prevention (Thompson et al. 2010). However, Lp-PLA_2 is not recognized as a suggested or recommended biomarker of cardiovascular risk in some reports (Vittorini and Clerico 2008, de Backer 2009).

Statins decrease plasma concentrations of Lp-PLA_2 (Braun and Davidson 2010, Rosenson et al. 211, Saougos et al. 2007, Schaefer et al. 2005), a more pronounced decrease of Lp-PLA_2 can be reached by darapladib, a direct inhibitor of Lp-PLA_2 activity (Mohler et al. 2008, Wilensky and MacPhee 2009). However, no significant reduction of primary end point (cardiovascular death, myocardial infarction, or stroke) was found in STABILITY trial (White et STABILITY investigators 2014), with significant reduction of secondary end points (major and total coronary events). There is a lack of studies focused on the evaluation of the relationship among different statins and their influence on Lp-PLA_2 and clinical outcome. Lp-

PLA_2 can be considered an independent risk factor without direct relation to lipid profile. Therefore, it should be important to know whether clinical outcome is influenced by lipid profile improvement, Lp-PLA_2 decrease, or both.

We measured Lp-PLA_2 as a marker of unstable plaque in a group of patients admitted to the emergency department. Biomarkers were measured in the first blood sample immediately after admission. Final diagnosis was determined by cardiologists at admission or during hospital stay according to current guidelines; cardiologists used only TnI as an emergency biomarker during admission. A retrospective analysis of records from the hospital information system was made by an internist (author JF). Patients with ACS were treated *lege artis* including introduction of statin therapy at discharge from hospital. About two thirds of patients were not treated with statins before admission to hospital and these patients were significantly younger ($p<0.002$). Lp-PLA_2 was significantly higher in patients with ACS in comparison to patients without ACS, irrespective of statin therapy ($p<0.0001$). In other words, Lp-PLA_2 is always higher in ACS patients, either without statin treatment or with statins. Similar data are available in literature indicating an increased concentration of Lp-PLA_2 in patients with ischemic cardiomyopathy or coronary artery disease (Ali and Madjid 2009, Charniot et al. 2013). However, CRP failed to discriminate between these two groups of patients (N.S.). Concentrations of Lp-PLA_2 were lower in patients with statins, both in ACS and non-ACS group (Table 1, Figure 1). CRP was not significantly influenced by statin therapy, neither in ACS, nor in non-ACS patients (Figure 2), as was expected (Biasucci et al. 2010). This could be caused by the gene-environment interaction in high risk patients (Lorenzová et al. 2007). Lp-PLA_2 seems to be more vascular-specific biomarker than CRP and benefitial effect of statins is less connected to CRP concentrations (Tousoulis et al. 2013). It is interesting that concentrations of TnI at admission were lower in ACS patients on statin therapy. We can only speculate that the lower concentration of TnI is due to the beneficial

effect of statins and less extensive myocardial damage. At present, there are neither consistent data on the role of $LpPLA_2$ in ACS patients (Oldgren et al. 2007, Holst-Albrechtsen et al. 2013). Based on our results, Lp-PLA_2 seems to be an effective biomarker of possible plaque instability and rupture in ACS. It should be stressed, however, that the best prediction of worse clinical outcome in sense of acute coronary syndrome by means of Lp-PLA_2 was found in patients under 62 years of age while increased concentrations of Lp-PLA_2 in patients of 62–73 years does not necessarily mean a high risk of acute coronary syndrome (Table 2, Figure 3). Probable explanation for this age dependency is higher frequency of other concomitant diseases in older and frequently polymorbid patients with other risk factors. On the other hand, the probability of ACS is generally more promiment in the third age tertile. Therefore, the predictive power of Lp-PLA_2 possibly depends on age. Surprisingly, statin-treated patients with lower age (below 50 years) and lower Lp-PLA_2 (under 300 μg/l) displayed increased odds of ACS. High risk in these patients was probably due to the other serious risk factors (unrelated to the age), which led to the initiation of statin therapy.

We suppose that the decrease of cut-off value of Lp-PLA_2 in patients with statins will optimise discrimination between ACS and non-ACS. Our data show that increased concentration of Lp-PLA_2 is linked to the higher risk of plaque instability and risk of STEMI/NSTEMI development. In spite of lower Lp-PLA_2 concentrations in statin treated group, patients developed STEMI/NSTEMI (ACS+/statin+). Therefore, cut-off of Lp-PLA_2 for the risk assessment should be decreased in statin-treated patients. The exact value of cut-off depends on the sensitivity or specificity of the test; our estimate is based on ROC analysis. Without statins, the optimal cut-off to discriminate between ACS and non-ACS patients was 238 μg/l with sensitivity of 80% and specificity of 35%. In the statin-treated group, the cut-off of similar sensitivity was 194 μg/l (sensitivity 82%, specificity 35%), i.e. cut-off lower of 19%. Similar results were observed both in the literature and in another group of our patients

(Ali and Madjid 2009, Saougos et al. 2007, Schaefer et al. 2005), where Lp-PLA_2 was measured before and 3 months after introduction of statin therapy (primary prevention). The difference in Lp-PLA_2 concentration between the patients with coronary artery disease (CAD) and without CAD was found significant in another study (223 μg/l vs. 208 μg/l) (Charniot et al. 2013). Higher concentrations of Lp-PLA_2 in our patients are probably due to the population tested – mainly clinically overt ACS with differential diagnostic groups of patients with cardiac diseases.

To conclude, concentrations of Lp-PLA_2 were significantly higher in ACS patients than non-ACS patients. The highest Lp-PLA_2 was found in ACS patients without prior statin therapy. Statin therapy decreases Lp-PLA_2 both in ACS and non-ACS patients. However, concentrations of Lp-PLA_2 in ACS patients were significantly higher even in statin therapy in comparison to non-ACS patients. Age is a significant modifier of the prognostic efficiency of Lp-PLA_2 in men and women, best predictive power for the risk of acute coronary syndrome was found in patients up to 62 years. Statin therapy modifies cut-off values of Lp-PLA_2 and lower cut-off values (of about 20 - 25%) will increase the efficiency of Lp-PLA_2 in the diagnostic process. Lp-PLA_2 seems to be an effective biomarker of plaque instability, but statin therapy and age should be taken into consideration while interpreting Lp-PLA_2 concentration.

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Table 1

Description of measured variables in four groups of patients. See text for details. Significance is given based on the Kruskal-Wallis test for the entire group. Pairwise comparison of subgroups according to Conover revealed significant age differences ($p<0,05$) for subgroups 1 vs 2, 1 vs 4, and 3 vs 4, respectively. Similarly for Lp-PLA_2, all pairs of subgroups were significantly different except for pairs 2 vs. 4. For TnI, all pairs of subgroups were significantly different except for pairs 3 vs. 4.

	Groups of patients				Signi-ficance
	1 ACS+/STATIN- (N=99)	2 ACS+/STATIN+ (N=60)	3 ACS-/STATIN- (N=107)	4 ACS-/STATIN+ (N=86)	p
Age	63.0 (52.3–73.8)	68.5 (60.5–76.0)	64.0 (54.3–75.0)	70.0 (61.0–77.0)	<0.01
Lp-PLA_2 (µg/l)	348.8 (248.3–445.1)	239.6 (203.2–302.4)	280.0 (221.9–345.2)	224.9 (188.2–264.6)	<0.0001
CRP (mg/l)	6.7 (2.3–19.0)	4.5 (1.5–10.8)	6.3 (1.8–24.1)	5.1 (1.7–21.3)	N.S.
TnI (µg/l) [a]	1.14 (0.06–8.09)	0.10 (0.03–0.99)	0.04 (0.03–0.12)	0.03 (0.03–0.09)	<0.0001

[a] used by cardiologists during diagnostic process

Table 2

Results of logistic regression analysis, where clinical outcome (ACS or non-ACS) was taken as dependent variable in a model consisting of age, sex, and Lp-PLA_2 as independent variables. Calculations are given for entire group of patients and for terciles of age. The chi-square test was used for overall model fit description.

Age (years)	Overall model fit	Percent of correct classification	AUC (95% confidence interval)
All range (see Table 1)	p<0.0001	62.4	0.659 (0.607–0.708)
<62	p<0.0001	65.6	0.725 (0.638–0.801)
62–73	N.S.	60.0	0.631 (0.538–0.717)
> 73	p<0.04	65.8	0.611 (0.514–0.702) *)

*) Significantly different from AUC for patients of age under 62 years.

Figure 1

Lp-PLA_2 in patients with final diagnosis of ACS (STEMI or NSTEMI) or non-ACS with respect to statin therapy. All pairwise comparisons showed significant difference ($p<0,05$) for all groups except for pair 2 vs. 3.

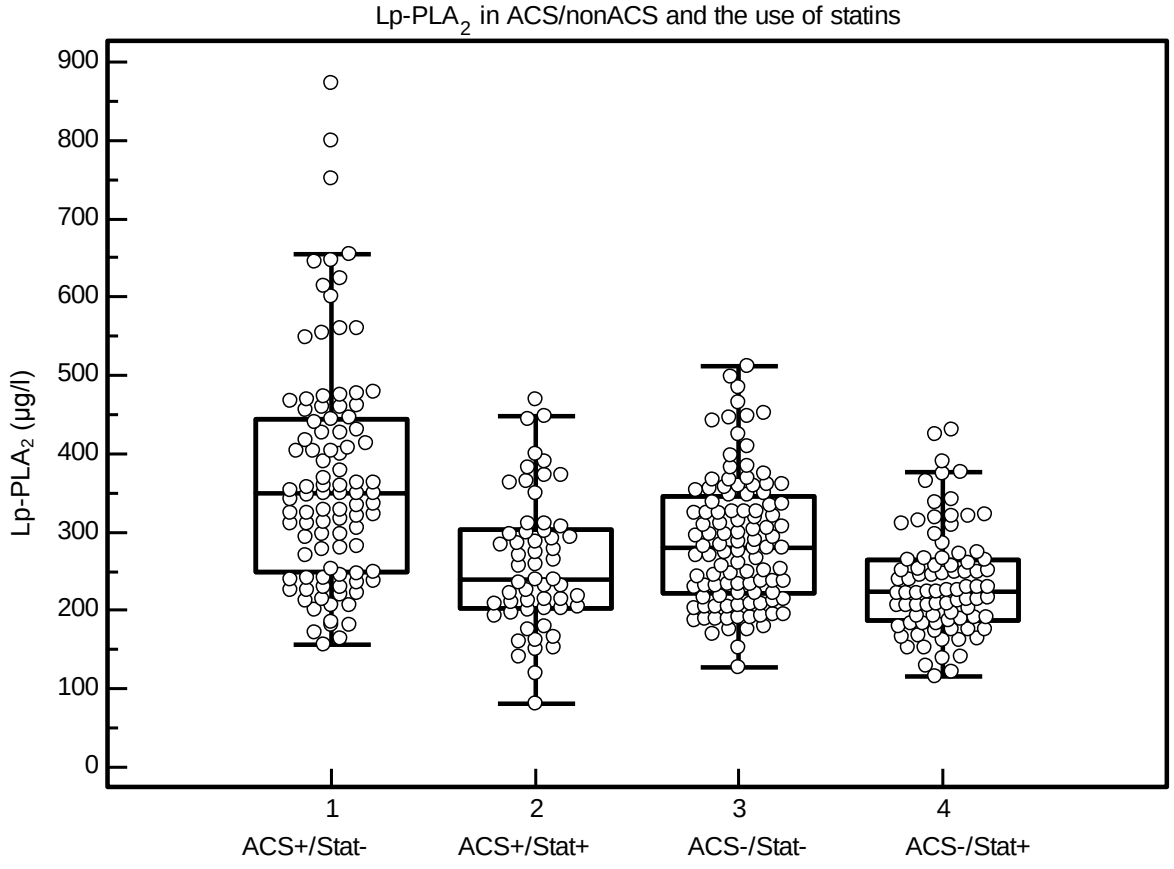

Figure 2

CRP in patients with final diagnosis of ACS (STEMI or NSTEMI) or non-ACS with respect to statin therapy. Not significant for all pairwise comparisons.

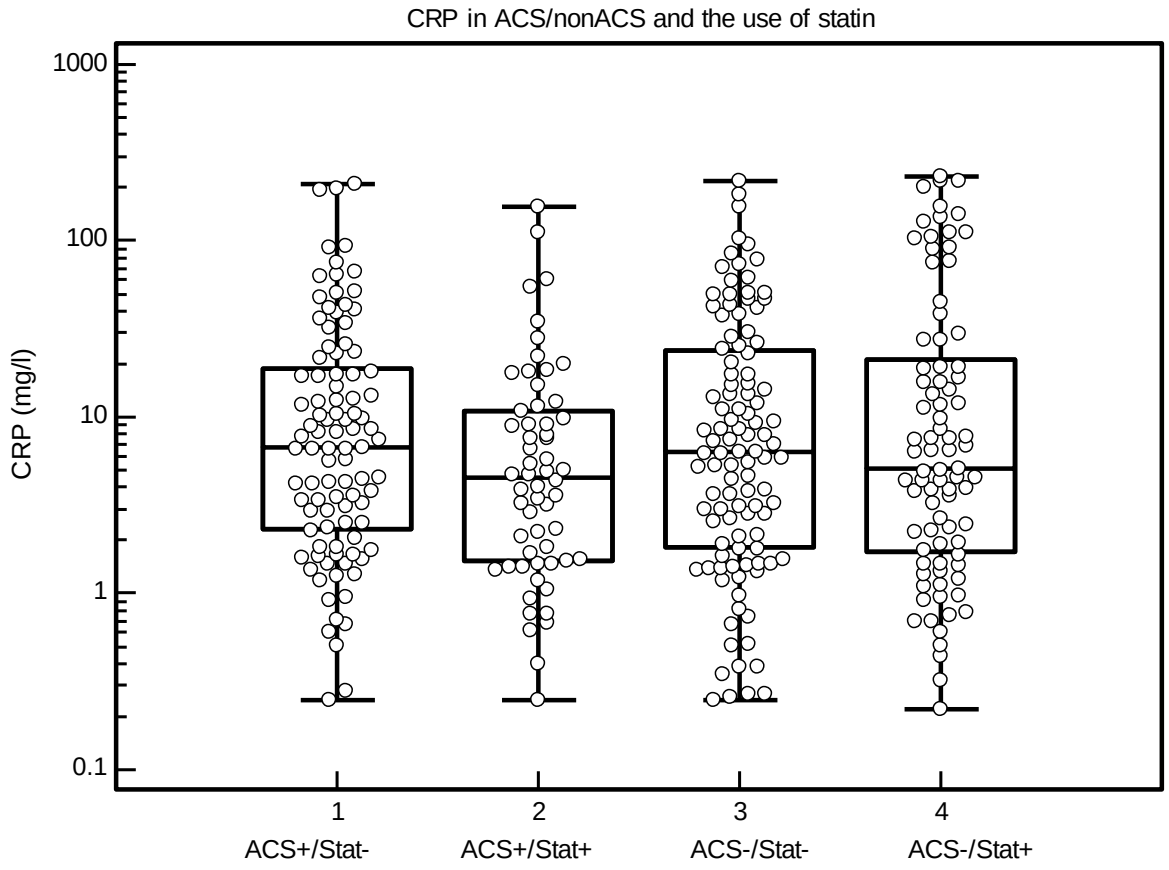

Figure 3

Interaction among probability of ACS, age, and Lp-PLA_2. Restricted cubic spline surface in two variable, each with k=4 knots. Log odds for ACS on vertical axis. Left panel – men (N=248), right panel – women (N=114).

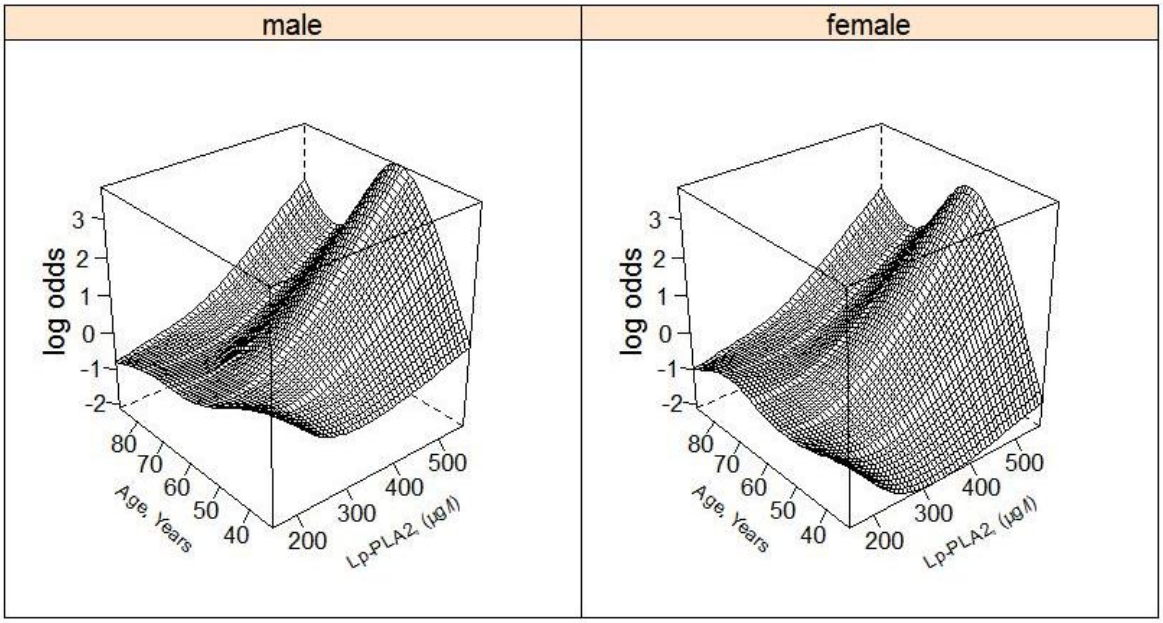

Figure 4

The influence of statins on the interaction among age, Lp-$PLA_{2,}$ and probability of ACS. Restricted cubic spline surface in two variable, each with k=4 knots. Log odds for ACS on vertical axis. Left panel – no statin therapy (N=206), right panel – statin therapy (N=146).