Low levels of IgM antibodies against phosphorylcholine are associated with fast carotid intima media thickness progression and cardiovascular risk in men

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A R T I C L E   I N F O

A B S T R A C T

Objective: Low levels of IgM anti-phosphorylcholine (anti-PC) increase the risk of cardiovascular events (CVE). Here we investigate the association of low anti-PC with the progression of carotid intima media thickness (C-IMT) and incidence of CVE in a large cohort of individuals at high risk of CVE, the IMPROVE, a prospective multicenter European study.
Methods: 3711 subjects (54–79 years) with at least three established cardiovascular risk factors were enrolled. Baseline serum levels of IgM anti-PC were measured by ELISA. Carotid ultrasound investigations were performed at baseline and after 15 and 30 months of follow-up. The risk of C-IMT progression and ischemic CVE associated with low anti-PC levels was tested by logistic regression and Cox regression analysis, respectively. Risk estimates were adjusted by center and conventional cardiovascular risk factors.
Results: 3670 study participants were included in the present analysis and 213 CVE were recorded during a 3 year follow up. Anti-PC levels (U/ml) were classified into quartiles [Q1≤ 40, Q2 >40-≤64, Q3 >64-≤102, Q4 >102]. In men, low levels of anti-PC (Q1) were associated with the highest (>90th) percentile of the fastest C-IMT progression, i.e. the segment showing the fastest progression over 30 months in the whole carotid tree, with an OR of 1.41 (95%CI, 1.02−1.9) and with an increased risk of CVE with a multivariable adjusted HR of 1.85 (95%CI, 1.1−3.1). No significant associations were found in women.

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Conclusions: Low anti-PC levels increase the risk of CVE in men. This effect may be partly mediated by a fast C-IMT progression.

1. Introduction

Phosphorylcholine (PC) is an ubiquitous antigen present on the surface of bacteria and a component of several phospholipids, phosphatidylcholine (PTC), the platelet activating factor and lysophospatidylcholine which constitute the cell membrane. Oxidation of these lipids is associated with an inflammatory response and with a conformational change that expose the PC epitope. PTC is also a constituent of the LDL, and PC is exposed on the surface of modified and oxidized LDL (ox-LDL) [1].

Serum IgM anti-PC levels are regulated by genetic and environmental factors [2] and participate in the natural and acquired immune responses [1]. Observational studies have shown that low serum anti-PC levels are associated with an increased risk of cardiovascular events (CVE) [3–6] in large Swedish cohorts. Further, we have also reported that anti-PC is inversely associated with atherosclerosis progression among hypertensive patients [7].

The mechanisms underlying the inverse relationship between anti-PC and risk of CVE have not been fully elucidated. It has been suggested that high circulating levels of anti-PC counteract the pro-inflammatory and pro-atherosclerotic effects of PC on the vessel wall [8].

In the present study we sought to investigate the association between low serum anti-PC levels and progression of carotid intima media thickness (C-IMT), an established measure of subclinical atherosclerosis, and risk of CVE in a large European multicenter study, the IMPROVE, designed to investigate the role of C-IMT progression on the prediction of CVE in individuals with multiple cardiovascular risk factors.

2. Material and methods

2.1. Study population

A detailed description of the IMPROVE study has been reported elsewhere [9–11]. Briefly, 3711 subjects, aged 59–74 asymptomatic for cardiovascular diseases, but with at least three established cardiovascular risk factors [men or women at least 5 years after menopause, dyslipidemia, hypertension, diabetes, smoking and family history of cardiovascular diseases], were recruited in five European countries, Finland ($n = 1050$, recruited in two centers in Kuopio), Sweden ($n = 533$, recruited in Stockholm), the Netherlands ($n = 532$, recruited in Groningen), France ($n = 501$, recruited in Paris) and Italy ($n = 1095$, recruited in Milan and Perugia). Study participants were followed up for 36 months, with visits at 15 and 30 months. At baseline, information regarding dietary habits, comorbidities and medications were collected and all participants underwent a physical examination. Ethnicity has been controlled by restricting the study to Caucasian subjects. A large biobank was established by blood sampling and storage ($-80\,^\circ$C) of whole blood, serum and plasma samples. Hs-CRP was measured as previously described [9].

For the present study, 41 individuals were excluded (carotid arteries could not be investigated, $n = 8$; discrepancy between recorded and genotyped sex, $n = 19$; missing serum samples, $n = 14$), leaving 3670 individuals for the analysis at baseline (men = 1756, women = 1914). At the 15 months visit 218 study participants did not participate [11], leaving 3452 individuals

available for the analysis (men = 1647, women = 1805); additional 198 individuals have been excluded from the present analysis because they did not have all the C-IMT measurements at the three time points (baseline, 15 and 30 months) leaving 3251 individuals (men = 1551, women = 1700) for the analysis at 30 months.

Incident CVE, namely angina pectoris, myocardial infarction, cardiovascular death, ischemic stroke, transient ischemic attack (TIA), coronary arteries and/or peripheral revascularization procedures, were recorded throughout the 36 months of follow up.

2.2. Ultrasonographic measures

Detailed descriptions of the ultrasonographic measures performed in the IMPROVE study have been fully reported elsewhere [9,10] and are summarized in the Supplemental Material.

2.3. Determination of antibodies against phosphorycholine (IgM anti-PC)

IgM anti-PC serum levels were only measured at baseline with an indirect non-competitive enzyme immunoassay (CVDefine, Athera Biotechnologies AB, Stockholm, Sweden) as previously described [3,4]. Briefly, the assay measures the total amount of IgM anti-PC in serum and is based on PCs covalently linked to bovine serum albumin (BSA) coated on 96-well microtiter plates. Serum samples were pre-diluted 1:100 in BSA-containing phosphate buffer before testing. Microtiter wells coated with PC-BSA were incubated for 30 min with 100 μl of diluted serum samples. After washing, the wells were incubated for 30 min with 100 μl of horse-radish labelled rabbit anti-human IgM conjugate. Thereafter the wells were washed and incubated in the dark with 100 μl substrate (TMB). Finally, after 10 min the color reaction was stopped by addition of 50 μl of 0.5 M H_2SO_4 and absorbance was measured at 450 nm with a reference wavelength of 620 nm (OD). All incubations were carried out at room temperature. IgM anti-PC levels in serum were expressed as arbitrary units (U/ml) determined from a calibrator curve containing 100, 50, 25, 12.5, 6.25 and 0 U/ml of IgM anti-PC.

2.4. Statistical analysis

Quantitative variables were reported as mean ± SD if normally distributed, otherwise as median (M) and interquartile range (IQTR). Categorical variables were reported as percentages.

Low levels of anti-PC have been associated with an increased risk of CVE in men [4], therefore the analyses were also stratified by sex. Anti-PC levels were divided in quartiles (Q) according to the distribution in the overall IMPROVE study at baseline. Anti-PC levels in the different Q were to ≤40 U/ml (Q1), >40 to ≤64 U/ml (Q2), >64 to ≤102 U/ml (Q3) and >102 (Q4). Individuals in Q1 were considered exposed to low anti-PC levels [3,4,8]. Differences in baseline C-IMT and total plaque area values as well as differences in the rate of C-IMT and total plaque area change after 30 months of follow up in individuals among the anti-PC quartiles were tested by Kruskal Wallis *H* test.

The correlation coefficient between serum levels of anti-PC, LDL-, HDL-cholesterol and CRP was calculated by the Spearman's rank correlation test.

To calculate the risk of suffering from a rapid C-IMT progression, a logistic regression analysis was used to estimate the relative risk, expressed as odds ratio (OR) and 95% confidence intervals (CI), of having a C-IMT progression above the 90th percentile when exposed to low anti-PC levels (Q1). The 90th percentile for the different progression measures at 30 months, were (mm/year) $IMT_{mean-progr}$ >0.05, $IMT_{max-progr}$ >0.22, $IMT_{mean-max-progr}$ >0.08, Fastest-$IMT_{max-progr}$ >0.35 and total plaque area$_{progr}$ >14.91 mm^2/year. Three different models of analysis were used: model 1: adjusted by participating center; model 2: as model 1 plus age, sex and the respective baseline C-IMT value; model 3: as model 2 plus hypertension, diabetes, hypercholesterolemia, smoking (defined as being an ever smoker versus having never smoked), abdominal obesity (defined as waist circumference >88 cm in women and >102 cm in men), baseline ICCAD and pharmacological treatment (statin, ACE-inhibitors, Angiotensin II receptor blockers, beta blockers and calcium-antagonists).

Cox proportional hazard regression models were used to estimate adjusted hazard ratios (HRs, 95% CI) of the combined endpoint (any CVE) associated with serum anti-PC levels. Adjustments were performed according to model 1, 2 and 3 as described above.

No correction for multiple comparisons was performed since baseline C-IMT measures and progression C-IMT measures were highly correlated [9,11,12].

The analyses were performed using SAS (v 9.01).

3. Results

Baseline demographic and anthropometric characteristics of incident cases and reference group in the IMPROVE study are reported in Supplemental Table I. Throughout the three years of follow up, 213 incident cases were recorded (3 sudden deaths, 34 acute myocardial infarctions, 48 with hospitalized angina, 32 ischemic strokes, 41 transient ischemic attacks, 13 coronary artery by-pass graftings, 4 peripheral artery by-pass graftings, 25 percutaneous angioplasty interventions and 13 new diagnosis of claudication).

Serum IgM anti-PC levels (U/ml) were higher in controls although the difference did not reach a statistical significance. Men had lower serum anti-PC levels than women [59 (36–97) vs 69 (43–102), $p < 0.0001$]. Study participants from Sweden had slightly higher serum anti-PC levels as compared to the study participants from the other centers [69 (42–112) vs 63 (39–101), $p = 0.01$]. As shown in Supplementary Table II, IgM anti-PC levels were in general lower in the different CVE subgroups as compared to the reference group, with the exception of the small group of women with a diagnosis of stroke.

No correlations were observed between circulating levels of anti-PC and LDL-cholesterol (correlation coefficient: 0.01, $p = 0.34$), HDL cholesterol (correlation coefficient: 0.02, $p = 0.16$) and Hs-CRP (correlation coefficient: 0.02, $p = 0.18$).

3.1. Serum anti-PC levels and C-IMT at baseline

Baseline C-IMT (IMT_{mean}, IMT_{max}, $IMT_{mean-max}$) and total plaque area values in the study participants stratified by anti-PC quartiles are reported in Supplemental Table III. IMT_{mean} and $IMT_{mean-max}$ baseline recordings (mm) were higher in individuals in the lowest anti-PC quartile as compared to the highest anti-PC quartile [IMT_{mean} 0.86 (0.7–1.0) vs 0.84 (0.7–1.0), $p = 0.02$ and $IMT_{mean-max}$ 1.21 (1.0–1.4) vs 1.18 (1.0–1.4), $p = 0.04$].

3.2. Serum anti-PC levels and C-IMT progression

No differences were observed among the anti-PC quartiles in the progression of C-IMT assessed by the three progression variables, $IMT_{mean-progr}$, $IMT_{max-progr}$ and $IMT_{mean-max-progr}$, and by the total plaque area $_{progr}$ at the 30 months of follow up visit (Supplemental Table IV).

In contrast, as shown in Table 1, we observed differences in the distribution of the Fastest-$IMT_{max-progr}$ values across the anti-PC quartiles. Overall the highest Fastest-$IMT_{max-progr}$ values were observed in the lowest anti-PC quartile. At the 30 months follow up visit, a Fastest-$IMT_{max-progr}$ (mm/year) of 0.14 (0.07–0.2) was observed in individuals exposed to low levels of anti-PC (Q1) as compared to a Fastest-$IMT_{max-progr}$ of 0.125 (0.06–0.2) in individuals exposed to anti-PC serum levels Q2-Q4 ($p = 0.01$) and to a Fastest-$IMT_{max-progr}$ of 0.12 (0.06–0.2) ($p = 0.03$) in those exposed to the highest anti-PC quartile (Q4). When differences were analyzed in men and women separately, we observed a similar trend although the results did not attain statistical significance.

3.3. Serum anti-PC levels and risk of C-IMT progression and CVE

To estimate the relative risk of C-IMT progression in the presence of low anti-PC serum levels, we analyzed the association of low anti-PC serum levels with C-IMT and total plaque area progression >90th percentile at the 30 months visit.

As shown in Table 2, low anti-PC serum levels were associated with an increased risk of being a fast progressor, i.e. of having a Fastest-$IMT_{max-progr}$ >90th percentile, with a relative risk of 1.29 (95%CI 1.0–1.7) in the fully adjusted model. Given the sex-related differences with regard to the effect of serum anti-PC levels on the atherosclerotic process, we have analyzed the association of low serum anti-PC levels with the risk of C-IMT progression separately in men and women. Low levels of anti-PC were associated with an increased risk of fastest progression in men with an OR of 1.41 (1.02–1.9) $p = 0.03$ in the fully adjusted model, while in women the effect was smaller and non-significant.

No differences in the risk of C-IMT progression were observed for the other progression measures across the anti-PC quartiles (Table 2).

To estimate the risk of CVE associated with low anti-PC serum levels, the CVE risk associated with anti-PC serum levels within

Table 1
Ultrasonographic recordings of C-IMT fastest progression after 30 months of follow-up (Fastest-$IMT_{max-progr}$) in the IMPROVE study and in men and women according to baseline anti-PC serum levels.

		Quartiles of baseline anti-PC serum levels				P values	P values
		Q1	Q2	Q3	Q4	Q1 vs Q2-Q4	Q1 vs Q4
Fastest-$IMT_{max-progr}$	All	0.14 (0.07–0.2)	0.13 (0.07–0.2)	0.13 (0.06–0.2)	0.12 (0.06–0.2)	0.01	0.03
	Men	0.15 (0.08–0.3)	0.14 (0.07–0.2)	0.16 (0.08–0.3)	0.14 (0.07–0.2)	0.38	0.32
	Women	0.12 (0.06–0.2)	0.11 (0.07–0.2)	0.11 (0.05–0.2)	0.11 (0.05–0.2)	0.12	0.22

Carotid IMT progression values are expressed as mm/year and are reported as median (IQTR). Anti-PC serum levels were divided into quartiles and differences among the anti-PC quartiles have been calculated by Kruskall Wallis *H* test.

Table 2
Risk of having an C-IMT and total plaque area progression >90th percentile during 30 months of follow up in IMPROVE with low (Q1) anti-PC serum levels as compared with high anti-PC serum levels (Q2-Q4), in all study participants, in men and women.

	Model 1	Model 2	Model 3
	OR(95%CI)	OR(95%CI)	OR(95%CI)
All			
$IMT_{mean-progr}$ (>0.05 mm/year)	1.09 (0.8—1.4)	1.04 (0.8—1.3)	1.06 (0.8—1.4)
$IMT_{max-progr}$ (>0.22 mm/year)	1.17 (0.9—1.5)	1.11 (0.8—1.4)	1.12 (0.9—1.4)
$IMT_{mean-max-progr}$ (>0.08 mm/year)	1.23 (0.9—1.6)	1.18 (0.9—1.5)	1.19 (0.9—1.5)
Total plaque $area_{progr}$ (>14.91 mm²/year)	1.09 (0.8—1.5)	1.05 (0.7—1.4)	1.09 (0.8—1.5)
Fastest-$IMT_{max-progr}$ (>0.35 mm/year)	1.38 (1.1—1.7)[a]	1.30 (1.01—1.7)[a]	1.29 (1.0—1.7)[a]
Men			
$IMT_{mean-progr}$ (>0.05 mm/year)	0.9 (0.6—1.3)	0.9 (0.6—1.3)	0.93 (0.6—1.3)
$IMT_{max-progr}$ (>0.22 mm/year)	1.21 (0.9—1.7)	1.22 (0.9—1.7)	1.24 (0.9—1.7)
$IMT_{mean-max-progr}$ (>0.08 mm/year)	1.20 (0.9—1.7)	1.22 (0.9—1.7)	1.22 (0.9—1.7)
Total plaque $area_{progr}$ (>14.91 mm²/year)	0.97 (0.7—1.4)	0.9 (07—1.4)	1.00 (0.7—1.5)
Fastest-$IMT_{max-progr}$ (>0.35 mm/year)	1.43 (1.04—1.9)[a]	1.4 (1.02—1.9)[a]	1.41 (1.02—1.9)[a]
Women			
$IMT_{mean-progr}$ (>0.05 mm/year)	1.32 (0.9—2)	1.27 (0.8—1.9)	1.25 (0.8—1.9)
$IMT_{max-progr}$ (>0.22 mm/year)	0.92 (0.6—1.4)	0.90 (0.6—1.4)	0.90 (0.6—1.4)
$IMT_{mean-max-progr}$ (>0.08 mm/year)	1.15 (0.8—1.8)	1.12 (0.7—1.7)	1.15 (0.7—1.7)
Total plaque $area_{progr}$ (>14.91 mm²/year)	1.19 (0.7—2.0)	1.19 (0.7—2.0)	1.27 ((0.7—2.2)
Fastest-$IMT_{max-progr}$ (>0.35 mm/year)	1.15 (0.7—1.8)	1.13 (0.7—1.7)	1.11 (0.7—1.7)

[a] $P < 0.05$. Model 1: adjusted by participating center; Model 2: as model 1 plus age, sex and the respective C-IMT baseline value; Model 3: as model 2 plus hypertension, diabetes, hypercholesterolemia, smoking, abdominal obesity, baseline ICCAD and pharmacological treatment with statin, ACE-inhibitors, Angiotensin II receptor blockers, beta blockers and calcium antagonists.

the lowest quartile was compared to quartiles Q2-Q4 and to the highest quartile (Table 3). The presence of low anti-PC (Q1) was associated with an increased risk of CVE with an HR of 1.41 (0.9—2.1) as compared to the highest anti-PC quartile (Table 3). When the analysis was performed in men and women separately, the risk of CVE was significantly increased in men with low anti-PC levels with a multivariable adjusted HR of 1.85 and 95%CI (1.1—3.1), $p = 0.02$ as compared to the highest quartile. Risk estimates for coronary events (myocardial infarction, hospitalized angina, coronary artery by pass and percutaneous intervention, $n = 75$) were higher in men with low anti-PC levels. A similar trend was observed for cerebrovascular events (ischemic stroke and TIA, $n = 41$), but the result did not attain statistical significance. (Supplemental Table V. No significant association was found in women.

Finally, the subgroup of men with a Fastest-$IMT_{max-progr}$ >90th percentile and low (Q1) anti-PC serum levels ($N = 73$) experienced the highest risk of CVE with an HR of 2.72 and 95%CI (1.2—6.2), $p = 0.01$, after adjustment for all potential confounders (model 3), as compared to men with Fastest-$IMT_{max-progr}$ values below the 90th percentile and higher anti-PC levels (Q2-Q4) ($N = 955$).

3.4. Serum anti-PC levels and the risk of C-IMT progression and CVE in different age groups

To investigate the effect of age, the IMPROVE study participants were stratified according to their age at baseline in two groups <65 ($n = 1927$) and ≥65 ($n = 1743$) years of age. The risk of fastest progression (Fastest-$IMT_{max- progr}$ >90th perc) associated with low serum anti-PC levels as well as the risk of incident CVE was higher in younger individuals (<65 year old) with an OR of 1.64 and 95%CI (1.1—2.3), $p = 0.008$ and an HR of 1.8 (0.9—3.4), $p = 0.06$, respectively.

No significant association with fast progression and risk of coronary events was found in individuals older than 65 years of age.

4. Discussion

In the present study we have investigated the association of anti-PC with the progression of C-IMT and the risk of CVE. Our results provide additional evidence that serum anti-PC levels represent an emerging, independent biomarker for cardiovascular diseases [13].

A former analysis of the determinants of baseline C-IMT values performed in the IMPROVE study has shown that a north to south gradient in latitude, and the dependent differences in the genetic background as well as in dietary habits, corresponds to a gradient in C-IMT [9] with the highest C-IMT values observed in Finland and the lowest in Italy. The differences in C-IMT values at baseline observed across the anti-PC quartiles are possibly related to genetic and environmental factors associated with a favorable cardiovascular risk profile that influence both lower C-IMT values and high serum anti-PC levels.

Changes of C-IMT over time may represent a relevant biomarker to estimate the progression of the atherosclerotic process as well as to evaluate the response to treatment. The mechanisms underlying the progression of C-IMT have not been fully elucidated to date. Serum cholesterol levels, systolic blood pressure and male sex predicted C-IMT progression in a 13 years follow up in the Tromso study [14], an increased ApoB/ApoA1 ratio and serum insulin levels were associated with C-IMT progression during 9 years follow up study in Swedish middle aged men [15] and low antioxidant serum

Table 3
Anti-PC serum levels at baseline and risk of CV events in the IMPROVE study and in men and women.

		Model 1	Model 2	Model 3
		HR (95% CI)	HR (95% CI)	HR (95% CI)
All	Anti-PC (Q1 vs Q4)	1.48 (1.0—2.2)	1.44 (0.9—2.0)	1.41 (0.9—2.1)
	Anti-PC (Q1 vs Q2-Q4)	1.20 (0.9—1.6)	1.11 (0.8—1.5)	1.15 (0.9—1.5)
Men	Anti-PC (Q1 vs Q4)	1.80 (1.0—3.0)[a]	1.76 (.0—2.9)[a]	1.85 (1.1—3.1)[a]
	Anti-PC (Q1 vs Q2-Q4)	1.33 (0.9—1.9)	1.32 (0.9—1.9)	1.38 (0.9—1.9)
Women	Anti-PC (Q1 vs Q4)	0.85 (0.4—1.7)	0.81 (0.4—1.6)	0.83 (0.3—1.5)
	Anti-PC (Q1 vs Q2-Q4)	0.77 (0.4—1.4)	0.74 (0.4—1.3)	0.75 (0.4—1.3)

[a] $P < 0.05$ Model 1: adjusted by participating center; Model 2: as model 1 plus age, sex and the respective C-IMT baseline value; Model 3: as model 2 plus hypertension, diabetes, hypercholesterolemia, smoking, abdominal obesity, baseline ICCAD and pharmacological treatment with statin, ACE-inhibitors, Angiotensin II receptor blockers, beta blockers and calcium antagonists.

levels have been associated with an increased C-IMT progression rate in the short term (18 months) in the Los Angeles Atherosclerosis Study [7,16,17]. In the present study, low serum anti-PC levels were not associated with C-IMT progression, when assessed with the commonly used measures of progression. In contrast, when the C-IMT progression was evaluated using a novel ultrasonographic measure, the Fastest-IMT$_{max-progr}$ [11], we observed that low anti-PC serum levels were associated with the highest Fastest-IMT$_{max-progr}$ values and with an increased risk of being a "fast IMT progressor". The fastest C-IMT progression takes into account only the carotid segment showing the fastest progression regardless of its localization within the carotid tree. One advantage of this newly proposed measure is the possibility to capture changes in the C-IMT over a relatively short time interval, thus overcoming the limitations associated with the conventional C-IMT progression measures [18]. When compared to the other composite progression measures, the Fastest-IMT$_{max-progr}$ has a higher yearly change and a better signal to noise ratio. These characteristics of the Fastest-IMT$_{max-progr}$ render this measure suitable for analysis of changes in C-IMT progression during a relatively short follow up time. As a down side, measurements of the Fastest-IMT$_{max-progr}$ require the acquisition of the entire carotid tree and a longer time to analyze the images [11].

Our current results in the IMPROVE confirm the inverse relationship between anti-PC levels and progression of atherosclerotic lesions that had been suggested by a previous study, performed by our group, in a small group of Swedish hypertensive patients where IgM anti-PC [7] and IgG1, but not IgG2, anti-PC [17] were inversely associated with C-IMT changes over a follow-up period of 4-years [7]. In the present study we have only investigated IgM anti-PC since the IgM isotype is stable over time, is highly correlated to the IgG1 isotype and has been widely investigated as a risk marker for CVE [17]. Of note, the assay used in the present study does not discriminate among the IgM-anti PC raised against the different PC antigens, but measures instead the total IgM-anti PC levels.

Low anti-PC levels, defined in this study as lower than 25th percentile as in previous studies from our [4,5] and other groups [19], were associated with an increased risk of CVE in the IMPROVE study. The current study represents the first investigation of serum anti-PC levels and risk of CVE in study participants from several European Countries, most of the other investigations [2–7,20] being performed in Swedish cohorts. The risk estimates associated with anti-PC levels were largely unaffected by the known cardiovascular risk factors indicating that anti-PC represents an independent biomarker for atherosclerosis related diseases.

Although we could not see differences in the distribution of the progression measures across the anti-PC quartiles in men, the unfavorable effect of low anti-PC on C-IMT progression as well as on the risk of CVE was only evident in men. This observation is consistent with former studies showing a sex difference in the risk of CVE associated with low serum anti-PC levels [4] and could possibly reflect the protective effect of higher serum anti-PC levels in women as compared to men [7] and the differences in the mechanisms underlying atherosclerotic diseases between men and women [21]. At the same time, in patients affected by systemic lupus erythematosus, low anti-PC levels were associated with an increased risk of CVE in women [22] and with the presence of carotid plaques in a cohort study where about 80% of the study participant were women [23]. These data indicate that the predictive role of anti-PC depends also on the cardiovascular risk of the populations under investigation.

The common and internal carotid IMT are commonly used as measures of subclinical atherosclerosis [24] and the common carotid IMT is an established predictor of CVE [20,25–27]. Data on the predictive value of C-IMT progression for CVE are controversial.

Progression of C-IMT was identified as a predictor of stroke in the Multi-Ethnic Study of Atherosclerosis [28], while a recent meta-analysis failed to prove a predictive role of C-IMT progression [29]. Consistently, data from the IMPROVE study, have recently shown that the conventional C-IMT and total plaque progression measurements do not add useful information for the prediction of CVE [11].

On the contrary, the Fastest-IMT$_{max-progr}$, independently predicted the risk of CV events and was superior to all the other conventional progression measures when sensitivity and reclassification analyses were performed [11]. The inverse relationship of serum anti-PC levels with the fastest progression and with the risk of CVE strengthens the hypothesis of a causal link between the Fastest-IMT$_{max-progr}$ and risk of CVE, observed in our previous study [11]. Given the biological role of anti-PC in the modulation of inflammatory and pro-atherosclerotic mechanisms, it is tempting to hypothesize that low levels of anti-PC may facilitate the progression of atherosclerosis eventually increasing plaque vulnerability and through this mechanism may increase the risk of CVE. Although we cannot prove directly this hypothesis from our data, the observation that low levels of anti-PC were associated with the highest risk estimate for CVE in individuals with the highest percentile of progression strongly support our hypothesis.

The risk associated with low anti-PC levels is higher in younger individuals (<65 years of age), in line with data showing that plaque composition varies at different ages and therefore also the mechanisms underlying plaque remodeling might differ across different age groups [30]. A corollary of this observation is that serum anti-PC levels may eventually be of particular use to identify individuals at risk for CVE before the age of 65.

In conclusion, our results demonstrate that serum anti-PC levels independently predict the risk of future CVE in a large European population of high risk individuals free of cardiovascular diseases. They also suggest that the observed increased risk of CVE is partly mediated by a fast progression of the atherosclerotic disease, measured by the progression of IMT in the carotid tree. On a broad perspective these results indicate that IgM anti-PC may represent a molecular target to ameliorate the immune and anti-inflammatory defense of the vessel wall.

Conflict of interest

None.

Acknowledgments

JF and UdF are named as co-inventors of patents owned by Athera Biotechnologies AB (cofounded by JF and UdF) relate to anti-PC and risk of cardiovascular diseases.

Appendix A. Supplementary data

Supplementary data related to this article can http://dx.doi.org/10.1016/j.atherosclerosis.2014.07.030.

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