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The interaction of persistent antiphospholipid antibodies positivity and cigarette smoking is associated with an increased risk of cardiovascular events: Cross-sectional and longitudinal analysis

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ABSTRACT

Background: The antiphospholipid antibody (aPL)-positivity was suggested as a nontraditional risk of coronary artery disease (CAD) and it was associated with cigarette smoking. The co-occurrence of them was usually reported in individuals with cardiovascular diseases. This study was to demonstrate their interaction on the increasing risk of cardiovascular events.

Methods and results: A total of 826 consecutive male individuals who underwent coronary angiography (CAG) /percutaneous coronary intervention (PCI) were prospectively followed and classified into three groups based on different smoking statuses. The current smoking subjects had the highest occurrence of aPL-positivity, including aCL IgM (20.1%) and aβ2GP1 IgM (15.5%). IgM isotype positivity was an independent risk factor of CAD in the multivariate model, OR: 2.70 (1.52–4.80) for aCL IgM and OR:2.50 (1.35–4.63) for aβ2GP1 IgM.The interaction of current smoking and IgM isotype positivity was significantly associated with increased risk of CAD, OR: 8.75 (4.59–16.66) for aCL IgM and OR: 8.78(4.28–17.98) for aβ2GP1 IgM. During about 3 years of follow-up, the smoking patients carrying persistent aPL positivity had the highest cumulative incidence of recurrent myocardial infarction and in-stent restenosis after CAD.

Conclusion: The interaction of current smoking and IgM isotype positivity was significantly associated with the increased risk of CAD, including positive aCL IgM and aβ2GP1 IgM. Cigarette smoking elevated the risk of subsequent cardiovascular events in the presence of IgM isotype positivity, including recurrent myocardial infarction and in-stent restenosis.

1. Introduction

Coronary artery disease (CAD) is usually characterized by the lipid metabolism disorder and accumulation of atherosclerotic plaques and is associated with numerous risk factors, including smoking, diabetes mellitus, hypertension, hyperuricemia, aging, obesity, physical inactivity, and immunological factors [1–4].

Cigarette smoking is considered a potent and modifiable risk factor that increases the risk of CAD and recurrent coronary artery events [5,6]. Smoking could double the risk of acute myocardial infarction and increase the risk of myocardial infarction and coronary death after incident myocardial infarction [6,7]. The severe cardiovascular side effects of smoking involve various pathophysiology processes, including increased blood pressure, dyslipidemia, increased oxidative stress,

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damaged endothelial function, and inflammation status [8,9], giving rise to plaque vulnerability and thrombosis. These pathological changes involve the immune system and immune responses, contributing to the initiation and exacerbation of atherosclerosis [10,11].

The antiphospholipid antibody (aPL) is considered an important non-traditional risk factor of CAD, including anti-cardiolipin (aCL), anti-β2-glycoprotein I antibodies (aβ2GPI) and lupus anticoagulant (LA), and its risk associated with CAD in part attributes to the accelerated atherosclerosis and recurrent thrombosis [12,13]. aPL activates platelets, monocytes, endothelial cells, and interleukins 6 and 8, promoting thrombus formation and resulting in CAD [14,15]. It is noteworthy that aPL-positivity is strongly linked to smoking commonly seen in patients with autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and antiphospholipid syndrome [16–19]. Their additive interaction has a stronger association with arterial and venous events in patients with systemic lupus erythematosus [17]. Interestingly, persistent aPL positivity can be detected in patients without autoimmune disease and shows enhanced subclinical atherosclerosis [13]. Whether the additive interaction between persistent aPL and smoking is associated with CAD and subsequent cardiovascular events in patients without autoimmune disease remains poorly understood. We hypothesized that smoking might increase the risk of developing CAD interacting with aPL-positivity, and related to the subsequent cardiovascular events. Thus, the present study aimed to investigate the association between the combination of smoking and aPL-positivity and cardiovascular events in a longitudinal study.

2. Method

2.1. Study population

During about 2 years (2018.01 to 2019.08), a total of 1410 consecutive subjects with clinical manifestations of suspected CAD who underwent CAG and/or PCI were prospectively recruited into this study. All subjects came from the Wenling Hospital Affiliated Wenzhou Medical University and were classified as the control group (never-smoking, NS-group), former-smoking group (FS-group), and the current-smoking group (CS-group) according to their smoking status. There were only five women with smoked; therefore, we excluded females. We also excluded subjects with infections, tumors, mental disorders, and overt autoimmune diseases, including confirmed antiphospholipid antibody syndrome. Subjects with previous strokes, homocysteinemia, congenital coagulation deficits and previous thrombosis were excluded. All subjects were informed of the details of this study and provided written informed consent. The study was performed following the Declaration of Helsinki. The Human Ethics Committee of Wenling Hospital Affiliated Wenzhou Medical University approved the study.

2.2. Smoking status

According to the patient's response to the question: 'Have you ever smoked daily for at least 1 year?' as elsewhere [17], smoking status was identified as current smoking, former smoking and never smoking. Current smoking was defined as regular smoking at enrolment, former smoking was defined as previously smoking but had quitted smoking before inclusion and never smoking was defined as never active or regular smoking. The tobacco product smoked by users was the usual combustible cigarette in our study. Smoking severity was usually involved in the degree of smoking exposure and nicotine craving degree of physiology and psychology. The details of cigarette smoking were recorded at enrolment, including the age of starting and stopping smoking and the number of cigarettes per day. The smoking index for each smoker was defined as the number of cigarettes smoked per day × the duration of smoking, and it was used to present the smoking exposure severity.

2.3. Clinical definition

CAD was defined as at least one coronary artery lesion stenosis ≥50% based on coronary angiography or coronary computed tomography angiography. Myocardial infarction (MI) included STEMI and non-STEMI, which were based on symptoms of myocardial ischemia, with or without ST-segment elevation on electrocardiogram, and increased serum levels of cardiac enzymes. In-stent restenosis was defined as stenosis of at least 50% of the luminal diameter between 5 mm from the proximal or distal edges of the implanted stent based on coronary angiography.

2.4. Follow-up

The details of cardiovascular events were performed by an independent medical group and they were blinded to this study. Subjects were followed up for recurrent myocardial infarction and in-stent restenosis until a loss to follow-up, or 31 January 2021, with a median follow-up period of 29 months (interquartile range 25–33).

2.5. Sample collecting and laboratory testing

The overnight fasting blood samples were collected and centrifuged at 5000 rpm for 5 min to separate plasma from cells. Each 0.5 mL plasma or cell was distributed into tubes and immediately stored at −80 °C until analysis. Levels of aPL including aCL IgA/IgG/IgM and aβ2GP1 IgA/IgG/IgM were measured using a chemiluminescence immunoassay analyzer (iFlash 3000, Shenzhen YHLO Biotech Co., Ltd.). Referring to the manufacturer's instructions, levels of aCL IgA/IgG/IgM ≥ 10 U/mL were considered positive and level of aβ2GP1 IgA/IgG/IgM ≥ 20 U/mL was positive. The limit of detection value of aCL IgA was 2.5 U/mL, aCL IgG was 1.0 U/mL, aCL IgM was 2.0 U/mL, and aβ2GP1 IgA/IgG/IgM was 2.0 U/mL, respectively. The screening and confirmation of LA detection were performed using the dilute Russell's viper venom time (dRVVT) and silica clotting time (SCT) method. To identify persistent aPL, another detection was performed at least 12 weeks apart and the result was positive on two or more determinations.

2.6. Statistical analysis

Continuous variables were presented as mean and standard deviation, and categorical variables were presented as proportions. The comparisons between the groups were performed using analysis of variance after the normal distribution test, and the independent-samples *t*-test was used to analyze the difference between the two groups. The χ^2 test was performed to evaluate categorical variables, and Fischer's exact was performed when a cell was ≤5. Logistic regression analysis was performed to estimate odds ratios (ORs) and 95% confidence intervals (CI) between smoking status and aPL, where never-smoking was considered the reference. The additive interactions were performed to evaluate the association of different smoking statuses and aPL positivity on the risk of CAD. As the reference suggested by Andersson et al., three indices were calculated, including relative excess risk due to interaction RERI = RR11 − RR10 − RR01 + 1, attributable proportion due to interaction AP = RERI/RR11, and synergy index SI = (RR11–1)/ [(RR01 –1) + (RR10–1)]. The 95% CI of RERI and AP did not include 0 or the 95% CI of SI did not include 1, the additive interaction was considered statistical significance [20]. Various logistic regression models were performed adjusting for age, body mass index (BMI), diabetes, and hypertension, as needed. Kaplan-Meier analysis was performed to assess the possible risk predictors using a log-rank test. All statistical analyses were performed using SPSS 20.0 software (Statistical Package for Social Studies, Version 20.0, SPSS Inc., Illinois, Chicago). $P < 0.05$ was considered statistically significant.

3. Results

3.1. The characteristics of subjects

A total of 826 participants were enrolled in this study, including 125 former smokers and 452 current smokers. Baseline characteristics were presented in Table 1. The mean age of subjects in the NS-group was 66.39 ± 12.44 years, higher than that of subjects in the FS-group (64.18 ± 10.27 years) and CS-group (63.34 ± 10.92 years) ($p = 0.003$). The proportion of hypertension was the lowest (54.0%) in CS-group ($p = 0.009$), but no difference was found between NS-group and FS-group. The proportion of CAD was the lowest (55.4%) in NS-group, and no significant difference between the FS-group (70.4%) and the CS-group (72.3%).

The information about smoking exposure was shown in Table 2. The age of smoking onset in the FS-group was 29.26 ± 8.55 years and it was younger compared to the CS-group ($p < 0.001$). The smoking cessation duration in the FS-group was 7.46 ± 5.59 years, ranging from 0.5 to 20 years. The number of cigarettes smoked per day in FS group was 27.92 ± 16.15 and no difference was found compared to CS-group (26.26 ± 14.03, $p = 0.297$). Compared to the FS-group, the smoking index was not significantly different from that of CS-group (741.4 ± 444.10 vs. 695.44 ± 483.56, $p = 0.339$).

In the CS-group, 91 subjects (20.1%) were aCL IgM-positive and the proportion was the highest among the three groups ($p = 0.003$). The ratio of subjects with positive aCL IgM was 14.4% in the FS-group and it was higher than that of the NS-group (10.4%). The proportion of subjects with positive aβ2GP1 IgM was also different between the three groups ($p = 0.013$). Seventy subjects with positive aβ2GP1 IgM were found in the CS-group; this was the highest and accounted for 15.5%. No difference of all the other antibodies was found among the three groups (Table 2).

Table 1
The characteristics of the included subjects.

Variable	Never Smoking Group ($n = 249$)	Former Smoking Group ($n = 125$)	Current Smoking Group ($n = 452$)	p
Age (years)	66.39 ± 12.44*	64.18 ± 10.27	63.34 ± 10.92	0.003
BMI (kg/m^2)	24.13 ± 3.36	24.06 ± 3.03	23.84 ± 3.18	0.576
ALT (U/L)	26.38 ± 17.22	26.21 ± 23.79	27.21 ± 20.85	0.823
AST (U/L)	38.24 ± 68.61	35.79 ± 58.33	34.31 ± 46.15	0.671
γ-GP (U/L)	38.29 ± 38.82	38.92 ± 37.19	45.34 ± 48.22	0.087
Serum creatinine (μmol/L)	85.94 ± 31.94	84.14 ± 25.78	80.99 ± 27.09	0.081
Urea nitrogen (μmol/L)	6.29 ± 2.93	6.24 ± 5.01	6.07 ± 2.84	0.681
Serum uric acid (μmol/L)	389.71 ± 110.49	387.27 ± 99.92	376.50 ± 95.19	0.215
Fasting glucose (mmol/L)	6.11 ± 2.64	5.92 ± 2.27	5.91 ± 2.25	0.538
HDL-C (mmol/L)	1.07 ± 0.27*	0.99 ± 0.26	1.02 ± 0.26	0.013
LDL-C (mmol/L)	2.79 ± 0.85	2.80 ± 0.84	2.83 ± 0.85	0.875
TG (mmol/L)	1.58 ± 0.84	1.66 ± 1.37	1.78 ± 1.07	0.065
TC (mmol/L)	4.33 ± 1.08	4.13 ± 1.03	4.25 ± 1.07	0.262
Reported history of Concomitant disease(n, %)				
Coronary artery disease	138(55.4)*	88(70.4)	327(72.3)	< 0.001
Hypertension	160(64.5)	81 (64.8)	244 (54.0) *	0.009
Diabetes mellitus	69(27.9)	29(23.6)	93(20.9)	0.108
Drug use (n, %)				
CCB	55(22.1)	29(23.2)	76(16.8)	0.120
ACEI/ARB	115(46.2)	56(44.8)	187(41.4)	0.440
β-blocker	137(55.0)	64(51.2)	184(40.7)	0.001
Antiplatelet agents	247(99.2)	124(99.2)	441(97.6)	0.195
Anticoagulants	52(21.0)	18(14.4)	93(20.6)	0.261
Statins	245(98.4)	124(99.2)	443(98.0)	0.654
Antihyperuricemia	20(8.0)	15(12.0)	33(7.3)	0.237
CRP(mg/L)	7.13 ± 18.06	5.87 ± 11.90	8.17 ± 21.56	0.482

BMI, Body mass index; ALT, alanine aminotransferase; AST, Aspartate aminotransferase;γ-GTP, γ - Glutamyl transpeptidase; HDL-C, High-density lipoprotein; LDL-C, Low-density lipoprotein; TG, Triglycerides; TC, Total cholesterol; LA, Lupus anticoagulant test.

Table 2
The severity of smoking exposure and antiphospholipid antibodies of the included subjects.

Variables	Never Smoking Group ($n = 249$)	Former Smoking Group ($n = 125$)	Current Smoking Group ($n = 452$)	p
Variables of smoking exposure				
Age at smoking onset (years)	/	29.27 ± 8.55	34.97 ± 14.19	< 0.001
Cigarettes smoked per day (n)	/	27.92 ± 16.15	26.26 ± 14.03	0.297
Smoking cessation duration (years)	/	7.46 ± 5.59	/	
Duration of smoking (years)	/	27.58 ± 10.20	28.35 ± 14.59	0.500
Smoking index	/	741.4 ± 444.10	695.44 ± 483.56	0.339
Antiphospholipid antibodies				
aCL IgA	8(3.2)	4(3.2)	11(2.4)	0.797
aCL IgG	6(2.4)	2(1.6)	8(1.8)	0.805
aCL IgM	26(10.4)	18 (14.4)	91(20.1)	0.003
aβ2GP1 IgA	3(1.2)	3(2.4)	4(0.9)	0.391
aβ2GP1 IgG	8(3.2)	3(2.4)	9(2.0)	0.602
aβ2GP1 IgM	20(8.0)	13(10.4)	70(15.5)	0.013
LA	27(10.8)	16(12.8)	50(11.1)	0.836

Smoking index = the number of cigarettes smoked per day × the duration of smoking; aCL, anti-cardiolipin; aβ2GPI, anti-β2-glycoprotein I antibodies; LA, Lupus anticoagulant test.

3.2. The association between different smoking statuses and aPL-positivity

Current smoking was associated with aCL IgM-positivity (OR: 2.79; 95% CI: 1.59–4.87) and aβ2GP1 IgM-positivity (OR: 2.78; 95% CI: 1.56–4.98), respectively. Current smoking remained a strong risk factor for aCL IgM and aβ2GP1 IgM-positivity after adjustment for other variables (Model 2, Table 3). No association was found between former smoking and IgM-isotype positivity (all $p > 0.05$). (Table 3).

3.3. The association between the smoking exposure and aPL-positivity

Because of the tight association between cigarette smoking and aPL-positivity, we suspected that the degree of smoking exposure might be causally related to IgM isotype positivity. We evaluated the association between variables related to cigarette smoking and aCL IgM and aβ2GP1 IgM-positivity. In multivariable-adjusted models, the smoking index was found to be significantly associated with IgM isotype positivity ($p < 0.05$). (Table 4).

3.4. aPL-positivity associated with smoking is a strong risk factor for CAD

Logistic regression analysis showed that aCL IgM and aβ2GP1 IgM were associated with the increased risk of CAD (OR: 2.45, 95% CI: 1.45–4.14 for aCL IgM; OR: 2.27, 95% CI: 1.29–4.01 for aβ2GP1 IgM) after adjustment for age, BMI, diabetes, hypertension, and smoking status. In further multivariable-adjusted model 2, aCL IgM and aβ2GP1 IgM remained strong risk factors for CAD (OR: 2.70, 95% CI: 1.52–4.80 for aCL IgM; OR: 2.50, 95% CI: 1.35–4.63 for aβ2GP1 IgM) (Table 5).

Table 3
Different smoking statuses are associated with aPL positive.

		aCL IgA	aCL IgG	aCL IgM	aβ2GP1 IgA	aβ2GP1 IgG	aβ2GP1 IgM	LA
Former smoking	Model 1	0.74(0.14–3.95)	0.77(0.14–4.18)	1.77(0.85–3.69)	4.20(0.34–51.62)	0.76(0.19–3.11)	1.17(0.51–2.73)	1.44(0.71–2.93)
	Model2	1.02(0.17–6.19)	0.74(0.11–4.79)	1.48(0.69–3.16)	3.31(0.33–33.75)	0.41(0.08–2.17)	1.07(0.45–2.54)	1.50(0.72–3.15)
Current smoking	Model1	1.04(0.34–3.19)	0.62(0.18–2.14)	2.79(1.59–4.87) **	3.12(0.29–33.29)	0.41(0.13–1.28)	2.19(1.19–4.01) *	0.95(0.53–1.69)
	Model2	1.51(0.43–5.27)	0.62(0.16–2.32)	2.78(1.56–4.98) **	2.45(0.30–20.18)	0.35(0.10–1.18)	2.29(1.23–4.28) *	1.04(0.57–1.91)

Presented as odds ratio (95% CI); *p < 0.05; **p ≤ 0.001;
Model 1: adjusted for age, BMI, diabetes and hypertension.
Model 2: Model 1 + HDL-C, LDL-C, TC, TG, and drug use including CCB, ACEI/ARB, β-blocker, antiplatelet agents, statins, anticoagulants, and antihyperuricemia.

Table 4
The association between smoking exposure and aPL positive.

	aCL IgM		aβ2GP1 IgM	
	OR (95% CI)	p	OR (95% CI)	p
Age at smoking onset	1.06(0.98–1.15)	0.101	1.07(0.98–1.18)	0.118
Duration of smoking	1.05(0.97–1.15)	0.182	1.06(0.96–1.17)	0.247
Cigarettes smoked per day	0.98(0.94–1.02)	0.352	0.98(0.94–1.02)	0.362
Smoking index	1.02 (1.0005–1.003)	0.007	1.02 (1.0003–1.003)	0.017

Presented as odds ratio (95% CI); adjusted for age, BMI, diabetes and hypertension.

Table 5
IgM isotype positivity is associated with the risk of CAD.

Variables	Model 1		Model 2	
	OR (95%CI)	p	OR (95%CI)	p
aCL IgM	2.45 (1.45–4.14)	0.001	2.70 (1.52–4.80)	0.001
aβ2GP1 IgM	2.27 (1.29–4.01)	0.005	2.50 (1.35–4.63)	0.003

Presented as odds ratio (95% CI).
Model 1: adjusted for age, BMI, diabetes, hypertension, and smoking status.
Model 2: model 1 + HDL-C, LDL-C, TC, TG.

3.5. The interaction between smoking status and IgM isotype positivity on the risk of CAD

To evaluate the additive effect, the interactions between IgM isotype positivity and smoking status were analyzed. The individuals were divided into four subgroups by their smoking status and IgM isotype. Compared with never smoking and aCL IgM negativity, the current smoking individuals combined with aCL IgM positivity had a significantly higher risk of CAD (OR: 8.75, 95% CI: 4.95–16.66) and the additive interaction was significant: RERI was 6.49 (1.08, 11.89), AP: 0.74, 95% CI: 0.56–0.92; SI: 6.14, 95% CI: 2.24–16.79. For the current smoking and aβ2GP1 IgM positivity individuals, the risk of CAD was higher than that of never smoking individuals combined with aβ2GP1 IgM negativity (OR: 8.78, 95% CI: 4.28–17.98) and the value of RERI: 6.33 (0.24–12.42), AP: 0.72(0.51–0.93); SI: 5.38(1.92–15.11) suggested a significant interaction. The significant interactions between former smoking and IgM isotype positivity were not observed. (Table 6).

3.6. The Kaplan-Meier survival analysis in smoking individuals combined with IgM isotype positivity

Of 826 included subjects, 9 (1.09%) participants suffered from thrombotic events that were no longer satisfied with the included criteria, and 58(7.02%) participants were lost during about 3 years of follow-up. A total of 758 participants were included in the follow-up study, including 238 never smokers, 116 former smokers and 432 current smokers. The overall median follow-up time was 29, IQR 25–33 months. Compared with never smoking and former smoking with IgM isotype negativity, the former smoking individuals combined with IgM isotype positivity had the highest risk of recurrent myocardial infarction and in-stent restenosis (Fig. 1). For current smoking individuals combined with IgM isotype positivity, the cumulative incidence of recurrent myocardial infarction was the highest compared to individuals with never smoking and current smoking with IgM isotype negativity, they also had an increased risk of in-stent restenosis (Fig. 2).

Table 6
The Interactions between smoking statuses and aPL positivity are associated with the increased risk of CAD.

Smoking status	IgM isotype	OR (95% CI)	Interactive effect
Former Smoking	aCL IgM		
–	–	1 (ref)	RERI =6.96 (−10.89–24.81)
–	+	0.86(0.38–1.93)	AP =0.79(0.35–1.23)
+	–	1.97(1.23–3.21)	SI =9.27(0.65–131.75)
+	+	8.80(1.15–67.29)	
Former Smoking	aβ2GP1 IgM		
–	–	1 (ref)	RERI =8.69(−7.50–24.88)
–	+	0.94(0.37–2.35)	AP =0.82(0.52–1.11)
+	–	2.01(1.25–3.24)	SI =10.19(1.22–85.30)
+	+	10.63 (2.30–49.08)	
Current Smoking	aCL IgM		
–	–	1 (ref)	RERI =6.49(1.08–11.89)
–	+	0.80(0.35–1.80)	AP =0.74(0.56–0.92)
+	–	2.47(1.75–3.48)	SI =6.14(2.24–16.79)
+	+	8.75(4.59–16.66)	
Current Smoking	aβ2GP1 IgM		
–	–	1 (ref)	RERI =6.33(0.24–12.42)
–	+	0.79(0.32–1.99)	AP =0.72(0.51–0.93)
+	–	2.65(1.89–3.72)	SI =5.38(1.92–15.11)
+	+	8.78(4.28–17.98)	

Adjusted for age, BMI, diabetes and hypertension, HDL-C, LDL-C, TC, TG, and drug use including CCB, ACEI/ARB, β-blocker, antiplatelet agents, statins, anticoagulants, and antihyperuricemia.

4. Discussion

The main result of this study was that IgM isotype positivity was an independent risk factor of CAD, and the interaction of current smoking and IgM isotype positivity was associated with the increased risk of CAD for patients carrying persistent aPL positivity. Furthermore, cigarette smoking had an increased risk of subsequent cardiovascular events in patients carrying persistent IgM isotype positivity, including recurrent myocardial infarction and in-stent restenosis.

The connection between cigarette smoking and aPL has been reported in a study [19]. Current smoking tended to be more closely associated with positive IgM isotype, and former smoking subjects had a higher incidence of positive aPL of IgG isotype in subjects with system lupus erythematosus [17]. Another study suggested that young women with cardio-cerebrovascular diseases were commonly current smokers with more frequent positive lupus anticoagulants; however, positive aCL

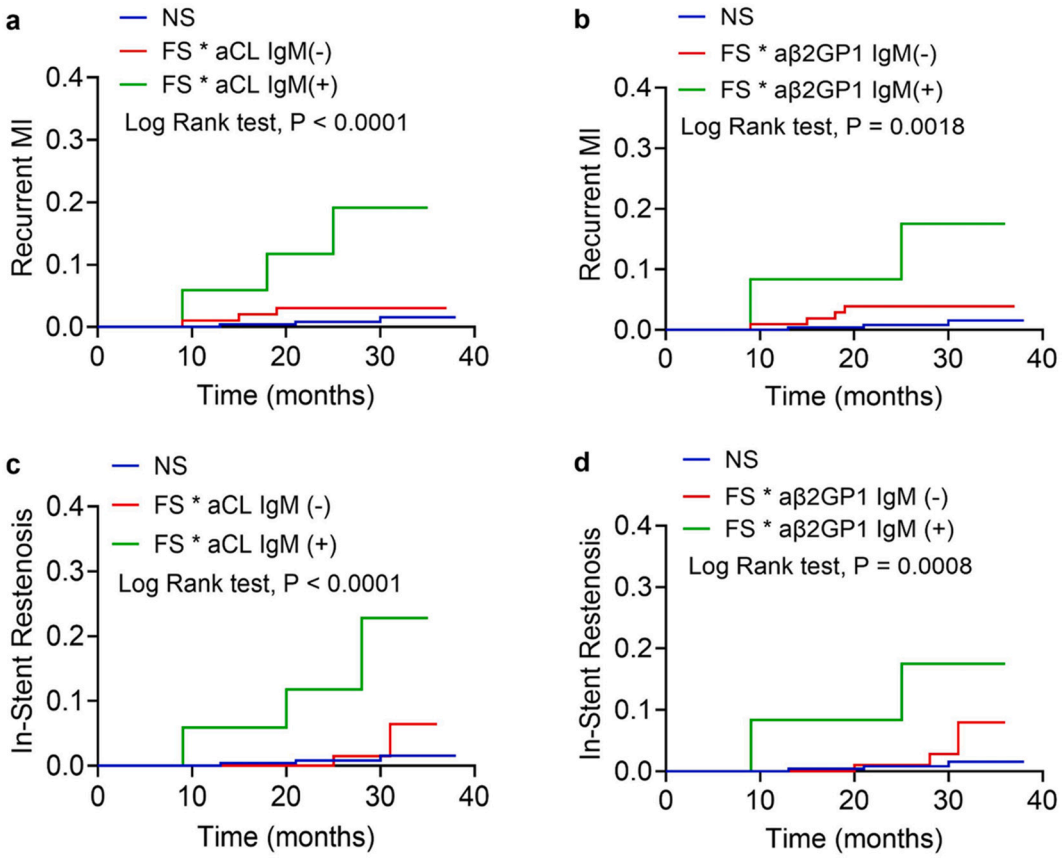

Fig. 1. Cumulative incidence curves for the recurrent cardiovascular events (including recurrent myocardial infarction and in-stent restenosis) of former smoking in the presence of IgM isotype positivity (green), competing for the risk of never smoking (blue) and former smoking combined with IgM isotype negativity (red), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

IgG and aβ2GP1 IgG did not show a significant distribution difference [21]. An earlier study did not find a significant association between cigarette smoking numbers per day and aCL mean values, nor between active and previous smoking; however, most subjects with high-positive IgG aCL (>100 GPL) were cigarette smokers [22]. It was acceptable that cigarette smoking would be associated with positive aPL [19]. Our findings suggested that the current smoking was associated with positive IgM isotype in subjects instead of IgA and IgG isotypes.

Several lines of evidence suggested that aPL was associated with coronary artery atherosclerosis, ischemic cardiovascular diseases, and ischemic stroke [23], but the categories of aPL associated with cardiovascular diseases remained to vary. IgG isotype and aβ2GP1 IgA were associated with subclinical atherosclerosis, and only aβ2GP1 IgG was associated with coronary artery calcification progression in healthy participants rather than IgA or IgM isotype [24]. aCL and aβ2GP1 isotypes were associated with ischemic stroke but not with CAD in a population that received health check-ups [25]. An earlier study suggested that aCL IgG was an independent risk factor for cardiac endpoints (cardiac death or nonfatal myocardial infarction); the risk doubled with simultaneous smoking in the Helsinki Heart Study population [26]. Our study found that IgM isotype was an independent risk of CAD in patients without autoimmune diseases, and the interaction between IgM isotype and current smoking was associated with an increased risk of CAD for the patients carrying persistent aPL positivity including aCL IgM and aβ2GP1 IgM, rather than former smoking. The aCL IgG and IgM were associated with increased stroke risk, and aCL IgM was particularly related to acute-phase responses to stroke [27]. Current smoking might display a triggering role in immunological responses, with initial atherogenic IgM production and later atherogenic IgG persistence [19]. However, the baseline serum levels of IgM isotype were no different from that of the 1 years follow-up in patients with CAD, but the IgG isotype showed an inconsistent change [28]. Thus, the persistent IgM isotype positivity might associate with cumulative smoking exposure and indicated an active immune response that induced acute cardiovascular events interacting with cigarette smoking.

Although the interaction of current smoking and IgM isotype positivity increased the risk of CAD was found in the cross-section analysis, whether aPL was an epiphenomenon of active cardiovascular events, especially for IgM isotype. We followed the recurrent myocardial infarction and in-stent restenosis events of individuals after receiving the coronary stent implantation treatment. Our study suggested that the interaction between IgM isotype and current smoking was not only associated with risk of CAD but was also associated with recurrent myocardial infarction and in-stent restenosis after an average follow-up of 29 months. In the healthy population, aCL IgG is an independent causal factor of myocardial infarction [26]. In a recent cross-sectional study, cardiovascular events and subclinical atherosclerosis were more frequent in the presence of aPL positivity including IgG and IgM isotype in a general population [29], suggesting that there was a tight relationship between cardiovascular events and aPL positivity rather than a simple epiphenomenon. A prospective study suggested that elevated aCL IgG and low aCL IgM were independent causal factors for recurrent cardiac deaths and nonfatal myocardial infarction, and they displayed a synergistic effect on increasing the risk of myocardial infarction [30]. The inconsistent association between aCL IgM and recurrent myocardial infarction was reported after an average follow-up of 18 months [31], but it did not concern the interaction of the aCL IgM and smoking. Interestingly, whether current smoking or former smoking, they both significantly increased the risk of recurrent cardiovascular events (recurrent myocardial infarction and in-stent restenosis) in subjects with

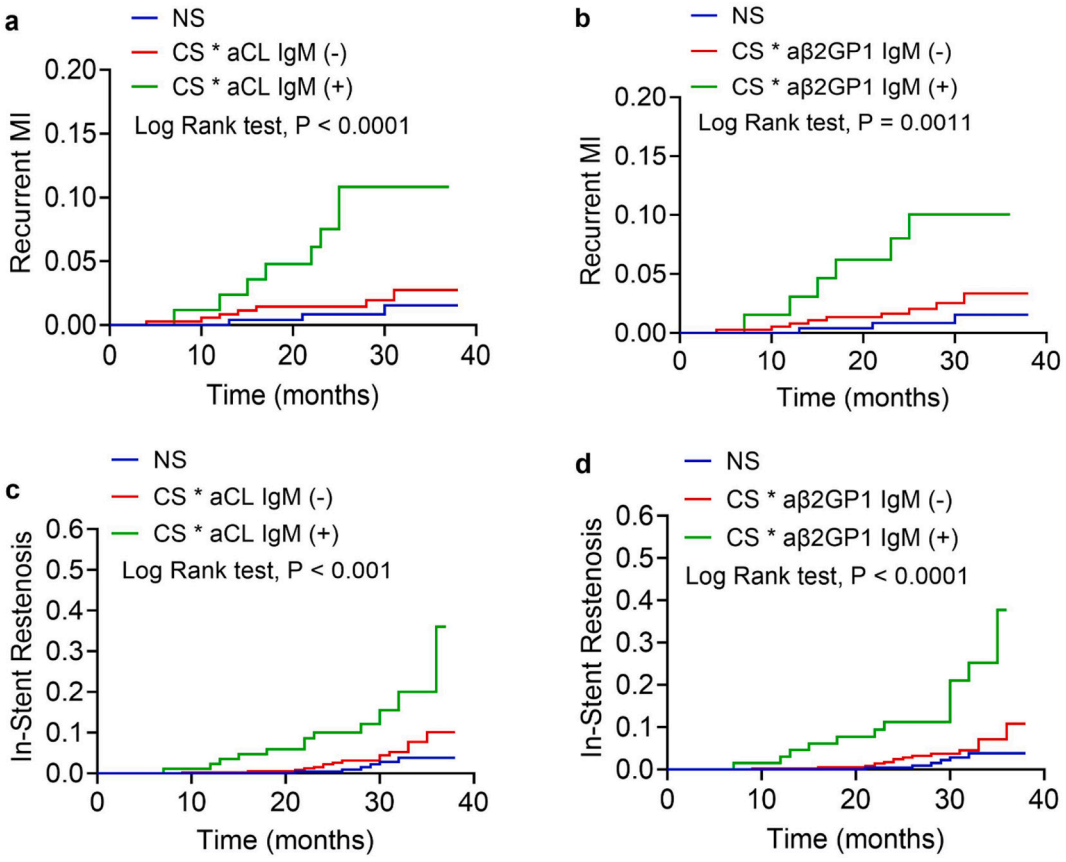

Fig. 2. Cumulative incidence curves for the recurrent cardiovascular events (including recurrent myocardial infarction and in-stent restenosis) of current smoking in the presence of IgM isotype positivity (green), competing for the risk of never smoking (blue) and current smoking combined with IgM isotype negativity (red), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

IgM isotype positivity in our study. Current smoking was confirmed to increase the risk of recurrent myocardial infarction within 1 year after PCI in both STEMI and NSTEMI subjects, and former smoking was associated with the risk of recurrent myocardial infarction within 1 year after PCI in NSTEMI subjects [32]. Smoking cessation usually brings intuitive beneficial effects. However, the smoking cessation duration was a crucial factor in whether beneficial effects displaying, short-term cessation did not bring a beneficial effect on coronary artery disease compared with never smoking [33]. And further, the cumulative impaired effect of cigarette smoking duration and intensity (number of cigarettes smoked per day) sustained even after smoking cessation for up to 20 years, especially for the harmful immune responses and coronary heart disease [34,35]. A clear association was noted between cigarette smoking and recurrent myocardial infarction. The risk of recurrent myocardial infarction was increased significantly for the individuals with smoking combined with IgM isotype positivity in statistical terms.

Of the recurrent myocardial infarction, approximately 25% of subjects were associated with in-stent restenosis [36]. In our study, a total of 38 subjects were in-stent restenosis and 10 subjects of them were recurrent myocardial infarctions, accounting for 26.32%, which was somewhat close to the above study. An early study reported that the incidence of in-stent restenosis was significantly higher in acute myocardial infarction subjects with aCL IgG < 40 than that of subjects with aCL IgG > 40 during the 12 months of follow-up [37]. Although not much is known about the association between the IgM isotype and in-stent restenosis, the association between IgM isotype and coronary artery restenosis after percutaneous transluminal coronary angioplasty (PTCA) has been concerned [38,39]. However, the pathophysiologic mechanisms of in-stent may be different from restenosis after PTCA. Now, new insight into the association between autoimmune diseases and in-stent restenosis was proposed, including rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid-antibodies syndrome, inflammatory bowel diseases, and others [40]. Although the subjects with overt immune diseases were excluded from our study, the active immune response associated with smoking exposure may indicate a potential risk of subsequent cardiovascular events.

There were several limitations in the present study. First, the study was only performed in males due to few female smokers; the results did not reflect the effect of sex on aPL-positivity, therefore, a further assessment needs to be performed in an expanded sample including females. Secondly, the benefit of smoking cessation is obvious in long term, but it may also need a long term for the impairment resulting from cigarette smoking to disappear completely. The sample in the former smoking group is too small to satisfy the subgroup analysis, the impact of smoking cessation duration on aPL-positivity and cardiovascular events needs to be furtherly investigated in a large clinical trial. In addition, the smoking severity is also involved in nicotine craving degree in physiology and psychology, which is not concerned in our study. Finally, the type of stents is also related to the cardiovascular events after PCI, such as bare-metal stents or drug-eluting stents, the impact of the type of stents on cardiovascular events should be taken into account in further study.

5. Conclusion

The interaction of current smoking and IgM isotype positivity was significantly associated with the increased risk of CAD, including positive aCL IgM and aβ2GP1 IgM. Cigarette smoking elevated the risk of subsequent cardiovascular events in the presence of IgM isotype positivity, including recurrent myocardial infarction and in-stent restenosis.

Authors' contributions

Wenhui Lin and Yanlong Liu conceived and designed the research. Jinzhong Xu, Yuncao Fan and Renfang Zhou wrote the paper and performed the experiments. Jianzhi Shao, Haihui Guo, Yunpeng Chen, Qizeng Wang supplied much advice and help to perform the experiments. Zhibing Dong, Mengjia Li, Ying Chen performed the statistical analysis and supplied advice. Shuangshuang Wang, Tian Jiang supplied technology. Yanlong Liu edited the final manuscript.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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