International Journal of Cardiology 274 (2019) 1–6

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Subclinical atherosclerosis in asymptomatic carriers of persistent antiphospholipid antibodies positivity: A cross-sectional study

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ARTICLE INFO

Article history:
Received 20 December 2017
Received in revised form 20 April 2018
Accepted 4 June 2018
Available online 5 June 2018

Keywords:
Antiphospholipid antibodies
Atherosclerosis
Risk factors

ABSTRACT

Background: Whereas the relationship between subclinical atherosclerosis and antiphospholipid syndrome (APS) has been widely investigated, little is known about subclinical atherosclerosis in asymptomatic carriers with isolated antiphospholipid antibodies positivity (APP).
Methods: Consecutive APP carriers, APS subjects and matched controls were enrolled. Intima-media thickness of the common carotid artery (CCA-IMT) and of the Bulb (Bulb-IMT) and the prevalence of carotid plaques were assessed in all enrolled subjects.
Results: A total of 104 APP carriers, 221 APS subjects, and 325 matched controls were recruited. As compared with controls, APP carriers and APS subjects showed a higher CCA-IMT (0.90 ± 0.24 vs 0.82 ± 0.12, $p = 0.014$ and 0.93 ± 0.42 vs 0.82 ± 0.12, $p < 0.001$, respectively), Bulb-IMT (1.10 ± 0.44 vs 0.95 ± 0.18, $p = 0.006$ and 1.22 ± 0.68 vs 0.95 ± 0.18, $p < 0.001$, respectively) and an increased prevalence of carotid plaques (33.7% vs 10.2%, $p < 0.001$ and 38.5% vs 10.2%, $p < 0.001$, respectively). These results were confirmed stratifying for antibody isotype, after excluding subjects with systemic lupus erythematosus or other autoimmune diseases and after adjusting for major clinical and demographic variables. CCA-IMT, Bulb-IMT and the prevalence of carotid plaques were higher in subjects with high-titer antibodies and progressively increased for an increasing number of positive antibodies.
Conclusions: Similar to APS subjects, APP carriers have enhanced subclinical atherosclerosis, a more severe disease being observed in the presence of high-titer antibodies and multiple antibodies positivity. These data argue for a strict monitoring of subclinical signs of atherosclerosis and of cardiovascular risk factors in asymptomatic APP carriers.

1. Introduction

Antiphospholipid antibodies (aPLs) are autoantibodies directed against plasma proteins bound to phospholipid membranes [1]. aPLs positivity (APP) is found in 1%–5% of the general population and it may be due to the positivity for lupus anticoagulant (LA) and/or anticardiolipin (aCL), and/or anti-β_2 glycoprotein-I ($a\beta_2$GPI) antibodies [2]. The antiphospholipid syndrome (APS) is an acquired autoimmune disease characterized by a persistent APP in subjects with history of venous and/or arterial thrombosis and/or recurrent miscarriage [3]. With a prevalence of 40–50 cases/100,000 persons [4], APS can occur in subjects with systemic lupus erythematosus (SLE) or other autoimmune rheumatic diseases (secondary APS) or in subjects without any concomitant autoimmune condition (primary APS) [5]. APS has been recognized as one of the most common acquired thrombophilic conditions [6] associated with an increased risk of both venous and arterial thrombotic events [7]. Some data suggest that venous thromboembolism accounts for >60% of vascular events associated with APS [8], arterial thrombosis being less commonly reported in APS subjects [9–11].

In recent years, there was a growing interest in the relationship between APS and subclinical atherosclerosis and a meta-analysis including 20 studies showed a more severe subclinical atherosclerosis in 668 APS subjects than in 678 matched controls [12]. However, all studies specifically enrolled APS subjects and no data are available about subclinical atherosclerosis in APP carriers. Being "per se" associated with an increased risk of atherosclerosis, a history of venous and/or arterial thrombosis

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https://doi.org/10.1016/j.ijcard.2018.06.010
0167-5273/

might have potentially masked the pro-atherogenic effect of aPLs in APS subjects [13, 14]. Thus, the aim of the present study was to evaluate subclinical atherosclerosis in APP carriers without venous/arterial thrombotic events.

2. Methods

During a 4-year period (January 2013–January 2017), consecutive subjects with a persistent positivity for aPLs (LA, IgG and IgM aCL, IgG and IgM aβ_2GPI) attending the Regional Reference Center for Coagulation Disorders of the Federico II University of Naples and the Lupus Clinic of the Department of Experimental and Clinical Medicine of the University of Florence were evaluated for enrollment in this cross-sectional study. Subjects were tested for the presence of aPLs following a thrombotic event or recurrent miscarriage, or in the frame of a primary prevention screening performed in pregnant women, in subjects with auto-immune disease, in the presence of thrombocytopenia or prolonged aPTT. The main inclusion criteria were the evidence of a persistent aPLs positivity (primary or secondary) in subjects of both genders and with an age ≥18 years. LA was identified by means of the diluted Russell Venom Time (dRVVT) and/or the aPTT-based test [15]. IgG and IgM aCL, IgG and IgM aβ_2GPI were tested using commercially available kits according to standardized procedures (EliA(TM)Cardiolipin and EliA(TM)β2-Glycoprotein - Phadia AB. Uppsala Sweden) [16]. IgG and/or IgM were defined positive when present in medium/high titer (i.e. >40 GPL or MPL) [17, 18]. Subjects with an aPLs titer ≤40 GPL or MPL were excluded. To only identify persistent aPLs (i.e. to avoid detection of transient antibodies), tests had to be positive on two or more determinations, at least 12 weeks apart [19]. IgG and/or IgM aCL as well as IgG and/or IgM aβ_2GPI were considered as single antibodies. Thus, single aPLs positivity refers to LA or IgG/IgM aCL or to IgG/IgM aβ_2GPI; double positivity refers to IgG/IgM aCL plus IgG/IgM aβ_2GPI or IgG/IgM aCL plus LA or IgG/IgM aβ_2GPI plus LA, and triple positivity refers to IgG/IgM aCL plus IgG/IgM aβ_2GPI plus LA. APS was diagnosed when a persistent positivity for aPLs was associated with a history of an objectively documented venous and/or arterial thrombosis and/or recurrent miscarriage. When clinical history, physical examination and diagnostic procedures (electrocardiogram and vascular ultrasound) excluded the presence of symptomatic and/or asymptomatic venous and/or arterial thrombotic events and of recurrent miscarriages, subjects were classified as APP carriers. Subjects without autoimmune disease, matched for age and gender and comparable for major vascular risk factors with the APP and APS groups were recruited in parallel, during the same time period from the hospital staff to serve as controls. All control subjects underwent a complete screening of aPLs and, in case of positivity, were excluded from the control group. To also take into account potential geographic differences, each control was enrolled in the same center as the matched case. A total of 196 cases (and matched controls) were enrolled in Naples and 129 cases (and matched controls) in Florence. Both for cases and controls, exclusion criteria were: lack of informed consent, age <18 years, positivity for aPLs documented in only one occasion, malignancy, hematologic diseases, unstable medical conditions, ongoing pregnancy. After informed consent, data about age, gender, height, weight, previous and/or current treatments and vascular risk factors were collected from cases and controls. Abdominal obesity, hypertriglyceridemia, hypercholesterolemia, hypertension, diabetes and impaired fasting glucose were defined according to the National Cholesterol Education Program (NCEP) criteria [20]. Both in cases and in controls, an ultrasound assessment of carotid intima-media thickness (IMT) was performed as previously described [21]. Briefly, after 5 min rest in the supine position, subjects underwent a bilateral carotid ultrasonography with a 7.5–12 MHz linear-array transducer and a duplex scanner. The ultrasound evaluation was performed longitudinally and transversally by using gray-scale and color-Doppler ultrasound imaging. The scan protocol requires the full-length visualization of the near and far wall of the right and left common carotid artery (CCA) and of the bulb in three different projections (anterior, lateral and posterior). IMT was measured in each of the three projections in CCA and bulb, and the presence of carotid plaques was defined as an IMT ≥1.3 mm. All the ultrasound examinations were performed by two operators (AS and PA) blinded as to the presence/absence of aPLs in each subject. The inter-operator reproducibility of the vascular measurements was evaluated in 25 subjects and the overall Pearson's r value for the IMT measurements was 0.93 ($p < 0.001$). The present study protocol was approved by Local Ethic Committees.

2.1. Statistical analysis

Statistical analysis was performed with the IBM SPSS 22 system (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation (SD). The *t*-test was performed to compare continuous variables. In case of values with a skewed distribution, Mann-Whitney *U* test was used to compare means. Correlations were assessed using the Pearson linear correlation coefficient (r). Categorical variables were expressed as % and analyzed with the χ^2 test. When the minimum expected value was <5, the Fisher's exact test was used. The absolute risk of carotid plaques presence in APS or APP subjects and controls was calculated as (number of subjects with carotid plaques) / (total number of subjects) in each group. The attributable risk was defined as (risk of carotid plaques presence in subjects with APS or APP − risk of carotid plaques presence in controls) / (risk of carotid plaques presence in subjects with APS or APP). To evaluate potential sources of heterogeneity, sensitivity analyses were performed: *a*) after stratifying the analysis according to antibody isotype; *b*) after stratifying APP carriers and APS subjects according to antibody titer defined as "high" when exceeding the median antibody titer calculated in our study population; *c*) after stratifying APP carriers and APS subjects according to the number of positive antibodies; *d*) after stratifying APS subjects according to the type of thrombotic event (arterial or non-arterial); *e*) only selecting APP carriers, APS subjects and controls without any cardiovascular risk factor; *f*) after excluding APP carriers and APS subjects with SLE or other autoimmune diseases. To adjust for all confounding variables and to evaluate the association between APS and APP with subclinical atherosclerosis, separate regression analyses were carried out, with CCA-IMT, Bulb-IMT and the presence of carotid plaques as the dependent variables and APP, APS, gender, age, obesity, body mass index, hypertension, hypercholesterolemia, total cholesterol, HDL cholesterol, LDL cholesterol, hypertriglyceridemia, triglycerides, diabetes/impaired fasting glucose, smoking habit, antiplatelet and anticoagulant treatment, statin and corticosteroids use and concomitant presence of SLE or other autoimmune diseases as independent variables. Multicollinearity effect was excluded by a stepwise approach with each variable included for $p < 0.05$ and excluded for $p > 0.1$. All the results are presented as 2-tailed values with statistical significance if p values <0.05. As to sample size, with a pre-defined difference in IMT between APP carriers and controls of at least 0.05 mm, 102 subjects for each study arm were needed to obtain an 80% power and a 5% α error.

3. Results

3.1. Study population

After excluding subjects with one or more exclusion criteria, a total of 325 subjects with a persistent positivity for aPLs were enrolled. Among them, 221 had a history of one or more venous and/or arterial thrombotic events and/or recurrent miscarriage and were classified as APS subjects. The other 104 were classified as asymptomatic APP carriers. A total of 325 controls matched with cases were recruited. Major clinical and demographic characteristics of the study population are reported in Table 1. APP carriers, APS subjects and controls were comparable for age and gender and for the prevalence of cardiovascular risk factors. As to the type of aPLs, IgG aCL and IgG aβ_2GPI were more often found in APS subjects than in APP carriers (79.6% vs 59.6% $p < 0.001$ and 45.7% vs 25.0% $p < 0.001$, respectively). No difference in the presence of all the other antibodies was found between APP carriers and APS subjects.

Treatment with antiplatelet drugs was reported in 31.2% of APS subjects and in 65.4% of APP carriers. A total of 21.2% of controls were receiving antiplatelet drugs in the frame of a primary cardiovascular prevention strategy. The use of vitamin K antagonists (with or without concomitant antiplatelet treatment) was reported by 61.0% of APS subjects. Only 2.9% of APP carriers were receiving vitamin K antagonists because of the concomitant presence of atrial fibrillation. Statins were more frequently used by APS subjects than APP carriers and controls, whereas corticosteroid use was equally distributed among study groups.

3.2. Subclinical atherosclerosis

Ultrasound examinations were performed after a median of 27 months (Inter-Quartile Range: 3–72) since aPLs detection. Interestingly, we found a direct and significant correlation between time from aPL detection, CCA-IMT ($r = 0.355$, $p < 0.001$) and Bulb-IMT ($r = 0.250$, $p < 0.001$).

The ultrasound assessment of carotid arteries showed a significantly higher CCA-IMT and Bulb-IMT in APP carriers and APS subjects than in controls (Table 2). Moreover, compared to controls, a significantly higher prevalence of carotid plaques was found both in APP carriers and in APS subjects, with an attributable risk of 69.7% for APP carriers and 73.5% for APS subjects (Table 2). A significantly increased carotid atherosclerosis was also confirmed when stratifying population according to antibody isotype (Data in brief Fig. 1).

Subjects with APS and arterial thrombotic manifestations showed the highest CCA-IMT and Bulb-IMT values, and the highest prevalence of carotid plaques, whereas no difference was found between APS subjects with venous thrombosis/recurrent miscarriages and APP carriers (Data in brief Table 1).

A higher CCA-IMT ($\beta = 0.241$, 95% CI: 0.052–0.228, $p = 0.002$), Bulb-IMT ($\beta = 0.239$, 95% CI: 0.091–0.358, $p = 0.001$) and prevalence of carotid plaques (OR: 4.14, 95% CI: 2.49–6.89, $p < 0.001$) was

Table 1
Clinical and demographic features of subjects with antiphospholipid syndrome (APS), carriers of antiphospholipid antibodies positivity (APP) and controls.

Variable	Controls (n = 325)	APP (n = 104)	APS (n = 221)	p value
Age	49.9 ± 12.5	48.6 ± 11.4	50.9 ± 12.9	1.000
Male gender	75 (23.1%)	21 (20.2%)	54 (24.4%)	0.699
Hypertension	92 (28.3%)	26 (25.0%)	67 (30.3%)	0.610
Hypercholesterolemia	132 (40.6%)	38 (36.5%)	95 (43.0%)	0.542
Total cholesterol	192.3 ± 38.5	190.6 ± 36.8	193.1 ± 40.5	0.860
HDL	54.7 ± 16.0	56.3 ± 17.1	53.9 ± 17.1	0.504
LDL	101.1 ± 35.3	98.6 ± 37.5	105.0 ± 39.2	0.281
Hypertriglyceridemia	20 (6.2%)	5 (4.8%)	7 (3.2%)	0.285
Triglycerides	94.4 ± 57.1	93.2 ± 57.4	89.7 ± 44.9	0.598
Obesity	122 (37.5%)	29 (27.9%)	83 (37.6%)	0.170
Body mass index	26.2 ± 5.5	25.5 ± 4.9	26.4 ± 5.6	0.345
Diabetes/impaired fasting glucose	22 (6.8%)	4 (3.8%)	20 (9.0%)	0.223
Smoking habit	113 (34.8%)	35 (33.7%)	76 (34.4%)	0.978
LA positivity[a]	–	60 (57.7%)	128 (57.9%)	1.000
aCL positivity (IgM)[a]	–	46 (44.2%)	85 (38.5%)	0.334
aCL positivity (IgG)[a]	–	62 (59.6%)	176 (79.6%)	<0.001
aβ_2-GPI (IgM)[a]	–	38 (36.5%)	63 (28.5%)	0.159
aβ_2-GPI (IgG)[a]	–	26 (25.0%)	101 (45.7%)	<0.001
Antibodies >80 GPL/MPL[b]	–	31 (29.8%)	62 (28.1%)	0.793
Systemic lupus erythematosus	–	41 (39.4%)	81 (36.7%)	0.626
Other autoimmune diseases	–	21 (20.2%)	40 (18.1%)	0.651
Arterial thrombosis	–	–	49 (22.2%)	
Venous thrombosis	–	–	136 (61.5%)	
Recurrent miscarriage	–	–	36 (16.3%)	
No antithrombotic treatment	256 (78.8%)	33 (31.7%)	17 (7.8%)	<0.001
Ongoing antithrombotic treatment				
APD	69 (21.2%)[c]	68 (65.4%)	60 (27.3%)	<0.001
VKA	0 (0%)	3 (2.9%)	95 (42.9%)	<0.001
APD + VKA	0 (0%)	0 (0%)	34 (15.6%)	<0.001
DAT + VKA	0 (0%)	0 (0%)	6 (2.6%)	0.003
DAT	0 (0%)	0 (0%)	9 (3.9%)	<0.001
Statins	140 (43.1%)	43 (41.3%)	121 (54.8%)	0.013[d]
Corticosteroids	–	42 (40.4%)	100 (45.5%)	0.410

LA: lupus anticoagulant; aCL: anticardiolipin antibodies; aβ_2GPI: anti $\beta_2$glicoprotein-I antibodies; APD: antiplatelets drug; VKA: vitamin-K-antagonists; DAT: dual antiplatelet therapy.

[a] Subjects presented >1 type of antiphospholipid antibodies concomitantly.

[b] IgG aCL and/or IgM aCL and/or IgG aβ2GPI and/or IgM aβ2GPI > 80 GPL or MPL.

[c] Control subjects receiving APD as cardiovascular primary prevention due to the presence of multiple cardiovascular risk factors.

[d] When comparing controls and APP p value was 0.820.

confirmed in APP carriers than in controls after adjusting for potential confounders by means of multivariate analyses. Similarly, multivariate analyses confirmed the association of APS with CCA-IMT (β = 0.523, 95% CI: 0.069–0.127, p < 0.001), with Bulb-IMT (β = 0.540, 95% CI: 0.125–0.223, p < 0.001) and with the prevalence of carotid plaques (OR: 7.33, 95% CI: 3.31–16.2, p < 0.001).

3.3. Sensitivity analyses

To test the impact of antibody titer on subclinical atherosclerosis, the whole study population was stratified according to antibody levels. In our study sample the median antibody titer was ≈80 GPL/MPL both for aCL (IgG and IgM) and for aβ2GPI (IgG and IgM). Thus, we defined high-titer aPLs in the presence of IgG and IgM aCL, IgG and IgM aβ_2GPI >80 GPL/MPL. As showed in Table 1, the prevalence of antibodies >80 GPL/MPL was similar in APS subjects and in APP carriers (28.1% vs 29.8%, p = 0.793). As compared with the 232 subjects with antibodies from 41 to 80 GPL/MPL, the 93 subjects with antibodies >80 GPL/MPL showed a significantly higher CCA-IMT (0.99 ± 0.47 vs 0.89 ± 0.31, p = 0.028) and Bulb-IMT (1.37 ± 0.86 vs 1.12 ± 0.43, p = 0.001). In keeping with this, carotid plaques were detected in 77 subjects (33.2%) with antibodies from 41 to 80 GPL/MPL and in 43 subjects (46.2%) with antibodies >80 GPL/MPL, with a corresponding OR of 1.73 (95% CI: 1.06–2.82, p = 0.031).

When stratifying APP carriers and APS subjects according to the antibody profile, we found a progressive increase in CCA-IMT, Bulb-IMT and prevalence of carotid plaques for increasing number of positive antibodies (Fig. 1). In detail, CCA-IMT, Bulb-IMT and prevalence of carotid plaques were significantly increased in subjects with triple-aPLs positivity, in those with concomitant presence of aCL and aβ_2GPI and in those with the isolated positivity of aβ_2GPI, as compared to controls (Table 3).

When repeating analyses only selecting subjects without any cardiovascular risk factor, we found a significantly higher CCA-IMT and Bulb-IMT with a marginally significantly higher prevalence of carotid plaques both in APP carriers and in APS subjects, as compared with controls (Data in brief Table 2).

Furthermore, to avoid the potential confounding effect of co-existing autoimmune diseases, all the analyses were repeated after excluding subjects with SLE or other autoimmune diseases and, as compared with controls, a higher CCA-IMT, Bulb-IMT and prevalence of carotid plaques were confirmed in APP carriers and in APS subjects (Data in brief Fig. 2).

4. Discussion

This is the first study showing that asymptomatic APP carriers have enhanced subclinical atherosclerosis, similar to APS subjects. Some previous data [22] on APP carriers suggested an increased risk of arterial events in this clinical setting, but no data were available on subclinical atherosclerosis. We report here an increased CCA-IMT and Bulb-IMT accompanied by an increased prevalence of carotid plaques both in APP carriers and in APS subjects as compared to controls matched for age, gender and major clinical and demographic variables. Whereas this issue has been widely studied in APS subjects, no information was available for APP carriers. However, in APS subjects the presence of a history of venous and/or arterial thrombosis, that are “per se” associated with an increased atherosclerosis, might have potentially masked the pro-atherogenic effect of aPLs [13, 14]. In contrast, having evaluated asymptomatic APP carriers, we are fully confident to have specifically evaluated the impact of antibody positivity on subclinical atherosclerosis.

Interestingly, all the results obtained in the primary analysis were confirmed by sensitivity analyses. We indeed investigated the impact of antibody titer on subclinical atherosclerosis, and we found that subjects with antibodies >80 GPL/MPL had a significantly higher CCA-IMT, Bulb-IMT and prevalence of carotid plaques than those with antibodies titer from 41 to 80 GPL/MPL. Furthermore, we found a progressive increase in carotid atherosclerosis for increasing number of positive antibodies and a maximal CCA-IMT, Bulb-IMT and prevalence of carotid plaques in subjects with triple-aPLs positivity, in subjects with concomitant positivity of aCL and aβ_2GPI and in those with the isolated positivity of aβ_2GPI.

These results are supported by previously published data [22] showing that a 10-unit increase in aCL IgG titer is associated with an OR of 1.07 (95% CI: 1.01–1.13) for arterial events and OR of 1.06 (95% CI: 1.02–1.11) for venous thrombosis. In addition, an OR of 1.5 (95% CI: 0.93–2.3) for arterial events and an OR of 1.7 (95% CI: 1.1–2.5) for venous thrombosis was found for each additional antibody detected. Overall, these data indicate that an increased titer of antibodies and multiple antibodies positivity are associated with an increased risk of both venous and arterial thrombotic events. Our findings are in keeping with such evidence, and also show a progressive increase in the severity of subclinical atherosclerosis in the presence of high-titer antibodies and multiple antibodies positivity.

The relationship between subclinical atherosclerosis and aPLs is complex and the traditional cardiovascular risk factors do not seem to fully explain the accelerated atherosclerosis found in this clinical setting. Inflammatory and immunological mechanisms were proposed to

Table 2
Common Carotid Artery Intima-Media Thickness (CCA-IMT), Bulb-IMT and prevalence of carotid plaques in carriers of antiphospholipid antibodies positivity (APP), subjects with antiphospholipid syndrome (APS) and controls.

Variable	Controls (n = 325)	APP (n = 104)	Difference vs controls	p vs controls	APS (n = 221)	Difference vs controls	p vs controls
CCA-IMT (mm)	0.82 ± 0.12	0.90 ± 0.24	MD: 0.08 mm (95% CI: 0.01–0.16)	0.014	0.93 ± 0.42	MD: 0.11 mm (95% CI: 0.06–0.17)	<0.001
Bulb-IMT (mm)	0.95 ± 0.18	1.10 ± 0.44	MD: 0.15 mm (95% CI: 0.03–0.27)	0.006	1.22 ± 0.68	MD: 0.27 mm (95% CI: 0.20–0.38)	<0.001
Plaques n (%)	33 (10.2%)	35 (33.7%)	OR: 4.49 (95% CI: 2.61–7.73)	<0.001	85 (38.5%)	OR: 5.53 (95% CI: 3.52–8.68)	<0.001

CCA-IMT: Common Carotid Artery Intima-Media Thickness; Bulb-IMT: intima-media thickness at the level of carotid bulb; plaques: defined as IMT ≥1.3 mm; APS: antiphospholipid antibodies syndrome; APP: antiphospholipid antibodies positivity; MD: mean difference; OR: odds ratio; 95% CI: 95% confidence intervals. Differences between APP vs APS: CCA-IMT p = 0.498; Bulb-IMT p = 0.101; plaques p = 0.403.

explain the relationship between aPLs and atherosclerosis [23]. Endothelial dysfunction, oxidative stress, increased expression of cell adhesion molecules, and platelet activation are common findings in the presence of aPLs [24]. Moreover, growing evidence suggests that APP is not only a marker of thrombophilia, but it might play a direct pathogenic role [25–31]. Some studies indeed argue for a pro-atherogenic role of aPLs and, in particular, of aβ_2GPI [32, 33]. β_2GPI is known to reduce the phagocytosis of oxidized LDL (oxLDL) by macrophages in the vessel wall [34]. In the presence of aβ_2GPI, this effect is inhibited and, in turn, an increased uptake of oxLDL by macrophages occurs, leading to accelerated atherosclerosis [35]. Accordingly, aβ_2GPI was found in atherosclerotic plaques obtained from human carotid endarterectomies [36], and β_2GPI was demonstrated to drive a Th1-related immune response in atherosclerotic lesions of primary APS subjects, thus fostering atherothrombosis [37]. Thus, this evidence might support the hypothesis of a specific pathophysiological role for aβ_2GPI in the atherosclerotic process. However, these results need to be confirmed in ad hoc designed studies.

Overall, our findings suggest that, similar to APS, premature atherosclerosis may be one of the main features of APP. The association with an increased subclinical atherosclerosis has a major clinical relevance being APP very often found in general clinical practice, with a prevalence of 1%–5% in the general population [2].

Moreover, the relevance of our results can be better understood when considering that subclinical atherosclerosis predicts cardiovascular events [38, 39] with a 43% increase in the risk of myocardial infarction every 0.163 mm increase in carotid IMT [40] and a 3-fold increased risk for cardiovascular and cerebrovascular events in APS subjects with increased IMT [41]. Overall, these data consistently support the hypothesis of an increased cardiovascular risk in asymptomatic APP carriers and might represent a preliminary evidence to extend to APP carriers the indication for a strict monitoring of subclinical signs of atherosclerosis and for the correction of cardiovascular risk factors previously suggested for APS subjects [42]. Therapeutic considerations should be basically similar to those applied in the general population. An aggressive control of traditional cardiovascular risk factors (i.e. hyperlipidaemia, hypertension, smoking, obesity, diabetes mellitus) should be performed by using both drug treatment and changes in lifestyle [43]. Aspirin prophylaxis may provide a potential benefit in subjects with aPLs by inhibiting the thrombogenic response [44] and treatment with statins, besides lowering total cholesterol levels, also improves endothelial function, increases the stability of atherosclerotic plaques, decreases oxidative stress and inflammation [45, 46]. Moreover, hydroxychloroquine could be useful in this clinical setting, since it has proved to be able to reduce the aPLs titer in APS subjects [47, 48]. However, studies specifically evaluating the effects of these treatments on cardiovascular outcomes in APP carriers are lacking and are urgently needed to address this issue [49, 50].

Some potential limitations of our study should be discussed. A potential confounding effect on results could be due to the underlying presence of SLE or other autoimmune diseases with a known pro-atherogenic effect. To overcome this potential source of bias, and specifically evaluate data on subjects with primary APS and APP, we repeated the analyses after excluding subjects with any autoimmune disease. This analysis confirmed an increased CCA-IMT and Bulb-IMT both in APP carriers and in APS subjects. Likewise, the presence of concomitant cardiovascular risk factors (hypertension, smoking, obesity, diabetes mellitus, and dyslipidemia) might have an impact on the severity of atherosclerosis, in the present setting. To provide a comprehensive overview on the relationship between APS and APP with subclinical atherosclerosis, we performed multivariate analyses to adjust for all the major markers of cardiovascular risk and we consistently confirmed the association of APP and APS with CCA-IMT, Bulb-IMT and prevalence

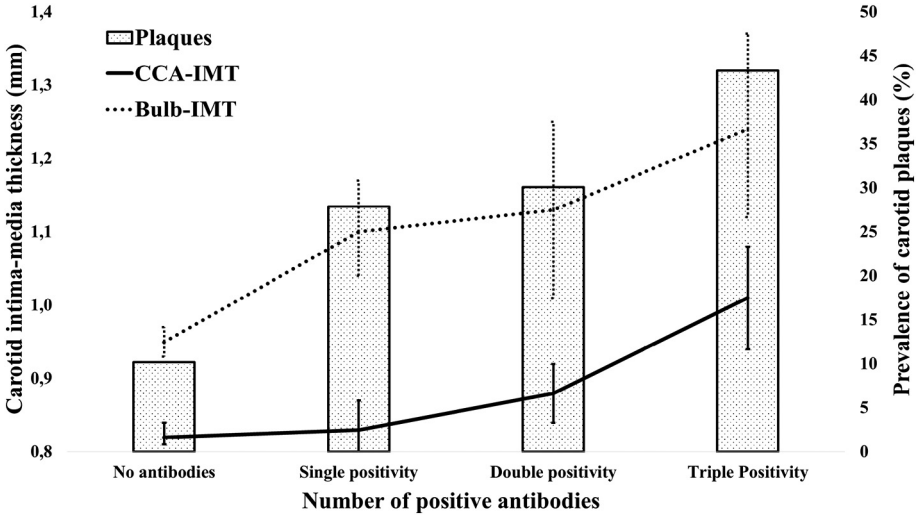

Fig. 1. Common Carotid Artery Intima-Media Thickness (CCA-IMT), intima-media thickness at the level of carotid bulb (Bulb-IMT) and prevalence of carotid plaques in carriers of antiphospholipid antibodies positivity (APP) and subjects with antiphospholipid syndrome (APS) stratified according to the number of positive antibodies. CCA-IMT: Common Carotid Artery Intima-Media Thickness; Bulb-IMT: intima-media thickness at the level of carotid bulb.

Table 3
Common Carotid Artery Intima-Media Thickness (CCA-IMT), Bulb-IMT and prevalence of carotid plaques in carriers of antiphospholipid antibodies positivity (APP), subjects with antiphospholipid syndrome (APS) and controls stratified according to antibody profile.

Antiphospholipid antibody profile			CCA-IMT mm	p*	Bulb-IMT mm	p*	Plaques %	p*
LAC	ACA	aβ2GPI						
+	+	+	1.01 ± 0.4	0.001	1.24 ± 0.7	<0.001	43.2%	<0.001
+	+	−	0.89 ± 0.2	1.000	1.13 ± 0.4	1.000	27.5%	0.084
+	−	+	0.84 ± 0.2	1.000	1.07 ± 0.5	1.000	25%	0.282
−	+	+	0.95 ± 0.3	0.003	1.22 ± 0.5	<0.001	42.9%	<0.001
+	−	−	0.80 ± 0.1	1.000	1.06 ± 0.2	1.000	27.3%	0.102
−	+	−	0.87 ± 0.3	1.000	1.13 ± 0.6	0.327	26.7%	0.245
−	−	+	0.93 ± 0.5	0.011	1.22 ± 0.6	0.062	37.5%	<0.001
−	−	−	0.82 ± 0.1	Comparator	0.95 ± 0.2	Comparator	10.2%	Comparator

* All p values are versus the control group without antibodies (i.e. Comparator).

of carotid plaques. In addition, all results were entirely confirmed in the sensitivity analysis specifically including subjects without any cardiovascular risk factor.

The presence of hemostatically active treatments might represent a significant source of bias in the present study. APS subjects were receiving a treatment with oral anticoagulants and/or antiplatelet drugs in approximately 92% of cases. Among asymptomatic APP carriers, 65% were receiving antiplatelet drugs as primary prophylaxis, all the others being without treatment. As to controls, an antiplatelet treatment was reported by about 20% of subjects in the frame of a primary cardiovascular prevention strategy. The extreme heterogeneity in treatment schedules made a subgroup analysis unlikely to be performed. However, we have included ongoing treatments in the multivariate analysis to overcome this potential bias, thus providing results adjusted for this co-variate. As a further point, it is important to consider that aPLs titer may fluctuate overtime. However, in the frame of periodic follow-up visits, all subjects underwent yearly aPL testing and, in all cases, antibodies resulted consistently positive, without significant changes.

In conclusion, we found that APP carriers, similar to APS subjects, have an increased subclinical atherosclerosis, a more severe disease being documented in the presence of high-titer antibodies and multiple antibodies positivity. These data strongly suggest that subjects with antiphospholipid antibodies positivity have an increased cardiovascular risk and that they may benefit from a careful screening for cardiovascular risk factors and ad hoc cardiovascular prevention strategies. Specifically designed studies are needed to establish the optimal management in these subjects.

Financial support information

No funds were received for this study.

Acknowledgements

Results of the present study were presented at the 118° National Annual Meeting of the Italian Society of Internal Medicine and awarded as best oral presentation.

Disclosure of conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

Authorship contributions

Matteo Nicola Dario Di Minno conceived and designed the study, performed the analysis, interpreted results and drafted the manuscript. Pasquale Ambrosino, Antonella Scalera, enrolled subjects, performed clinical and ultrasound examinations, acquired laboratory data and prepared the database for the analysis. Giacomo Emmi enrolled patient, performed clinical examinations, interpreted results and drafted the manuscript. Antonella Tufano, Giovanni Cafaro, Rosario Peluso, Alessandra Bettiol, Gerardo Di Scala and Elena Silvestri enrolled subjects, acquired laboratory data and prepared the database for the analysis. Domenico Prisco interpreted results and performed critical revision of the manuscript. All authors read and approved the final version of the manuscript.

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