

Antiphospholipid Antibodies and Atherosclerosis: Insights from Systemic Lupus Erythematosus and Primary Antiphospholipid Syndrome

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Abstract The issue of atherosclerosis in the antiphospholipid syndrome (APS) is receiving considerable attention within and without the autoimmune setting. Measurement of arterial intima media thickness (IMT) of is an easy and surrogate means of detecting subclinical atherosclerosis. This technique has been applied to patients with systemic lupus erythematosus (SLE) and primary APS in the attempt to unravel a possible association between antiphospholipid antibodies and premature atherosclerosis. The available data is reviewed in the light of the most recent atherogenic pathways that may differentially account for premature vascular disease in SLE and primary APS.

Keywords Antiphospholipid antibodies ·
Atherosclerosis · Systemic lupus erythematosus ·
Primary antiphospholipid syndrome

Introduction

Atherosclerosis is the end result of the effect of biochemical and coagulation pathways, of crosstalks amongst immune cells, and of oxidant/antioxidant imbalance on the endothe-

lium that thickens with age due to the persistence of the above factors. Indeed, atherosclerosis may be viewed as a low-grade immune-inflammatory disease [1]. This has led to investigations of the existence of atherosclerosis in autoimmune systemic diseases where inflammation of the vessel may associate with vascular morbidity and mortality in addition to that of the background disease [2].

Forerunner of these concepts was the increase risk of thrombosis in systemic lupus erythematosus (SLE) subsequently ascribed to the presence of antiphospholipid antibodies (aPL), hence to the antiphospholipid syndrome (APS) [3, 4]. Though atherosclerosis is an important contributor to arterial thrombosis, it was deceiving to consider thrombotic events as an endpoint for the detection of atherosclerosis in SLE and APS [3, 4]. The measurement of intima-media thickness (IMT) of carotid arteries by high-resolution sonography is a widely accepted means of detecting subclinical atherosclerosis [5] and has been applied to SLE and APS to evaluate the occurrence of premature atherosclerosis in these populations. Therefore, this review will focus essentially on those case-control studies assessing atherosclerosis in SLE and primary APS by means of IMT measurements and will include noncase-control studies assessing variables related to incident and/or progressive IMT.

Intima-media thickness in systemic lupus erythematosus

The first study showing a very high plaque prevalence and a thickened intima-media of carotid arteries of women with SLE appeared in the late 1990s: independent predictors of plaque were older age, higher systolic blood pressure, higher low-density lipoprotein (LDL), prolonged steroid

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Table 1 Intima–media thickness of common carotid artery in SLE studies

	SLE	CTR	<i>p</i> value	References
No.	197	197		Roman et al. [7]
IMT (mm)	0.61±0.16	0.67±0.14	<0.001	
Plaque (%)	37.1	15.2	<0.001	
No.	26 (CVD)	26		Svenungsson et al. [8]
IMT (mm)	0.66±0.15	0.59±0.12	0.001	
Plaque (%)	65	11	0.002	
No.	70	40		Jimenez et al. [9]
IMT (mm)	0.55±0.12	0.54±0.14	NS	
Plaque (%)	28.6	6	0.01	
Premenopause no.	14	17		Sato et al. [10]
Plaque (%)	36	0		
Postmenopause no.	25	22		Sato et al. [10]
Plaque (%)	44	9		
No.	51	47		Wolak et al. [15]
IMT (mm) ^a	1.36	1.37		
UB score	5.65±6.4	3.14±5.82	0.02	
No.	16	16		Rossi et al. [13]
IMT (mm)	0.66±0.08	0.62±0.13	NS	
Plaque (%)	NA	NA		
No.	26	26		Juvenile SLE
IMT (mm)	0.57	0.54	NS	Falaschi et al. [12] ^b
Plaque (%)				
No.	20	20		Juvenile SLE
IMT (mm)	0.48±0.04	0.454±0.04	NS	Bowser et al. [16]

SLE systemic lupus erythematosus, CTR control, IMT intima–media thickness, UB ultrasound biopsy

^aMean of IMT from R+L common carotid

^bIMT without proteinuria 0.56 mm (*n*=20) with proteinuria 0.61 mm (*n*=6)

treatment, and a history of coronary event. Independent predictors of IMT were older age, history of coronary event, and higher damage index [6].

Subsequent case–control studies have confirmed the higher plaque prevalence in SLE [7–11] (Table 1): independent predictors of plaque were age, disease activity [7, 9, 11] higher serum cholesterol or LDL level [7, 11], and a greater cumulative steroid dose [11]. However, plaque prevalence did not necessarily match IMT that was found greater only in two studies [8, 10]: higher triglycerides, lower high-density lipoproteins (HDL) amongst traditional cardiovascular risk factors and plasma fibrinogen, homocysteine, and lupus anticoagulant amongst the nontraditional cardiovascular risk factors associated with IMT in one study [8] and cumulative steroid dose in the other [10]. In juvenile SLE, nephrotic range proteinuria with dyslipidemia were associated with greater IMT than nonprotei-

nuric patients [11] whereas hypertension associated with greater IMT in SLE patients without cardiovascular risk factors [12]. A further survey on SLE specifically explored IMT in relation to inflammation (C-reactive protein), endothelial activation markers (vascular intercellular adhesion molecule, von Willebrand factor), and vascular remodeling (metalloproteinase and relevant inhibitor): they concluded that endothelial activation and vascular remodeling markers are not superior to traditional risk factors in their link with IMT [13, 14]. The remaining case–control studies (Table 1) found similar IMT in juvenile [15] and nonjuvenile SLE [16] though greater ultrasound biopsy score was found in the latter study compared to healthy controls [16].

The previous issues have been recently re-addressed in prospective studies: the first on 159 SLE patients (average interval between ultrasound of carotid arteries 34 months)

noted that only 28% of patients developed progressive plaques in carotid arteries that in multivariate analysis were independently predicted by plasma homocysteine concentration alongside age at diagnosis and disease duration [17]; the second on 217 SLE patients (average interval between ultrasound of carotid arteries 49 months) noted that only 27% of patients developed progressive plaques with a yearly IMT increment of 0.01 ± 0.03 mm (the latter not different from their control populations), independently predicted by serum creatinine. After controlling for age, lower socioeconomic status, lower HDL, higher triglycerides, smoking, and higher fibrinogen were amongst the traditional cardiovascular risk factors related to plaque progression whereas SLAM score, immune suppressant agents at baseline, and cholesterol-lowering medication were amongst the SLE-related cardiovascular risk factors related to plaque progression. In a multivariate model that controlled for traditional cardiovascular risk factors, serum C3 and immune suppressant agents at baseline predicted plaque progression [18].

Intima-media thickness in primary antiphospholipid syndrome

The detection of atherosclerosis in primary APS lagged behind that in SLE for the lack of suitable large populations: of the available studies, two did not find greater plaque prevalence and/or IMT [9, 19]. One of these correctly matched primary APS by age, sex, and other vascular risk factors to thrombotic patients without APS: having examined the abdominal aorta, carotid, and femoral arteries, the authors concluded that atherosclerosis was not a feature of their PAPS cohort [19]. At variance, a comparison between 28 PAPS patients and an equal number of age- and sex-matched nonthrombotic controls revealed greater IMT in the PAPS group [20]. A similar study on 14 primary APS and 36 controls yielded a similar result [21]. In our most recent update, we detected greater IMT in primary APS patients over 40 years of age compared to younger primary APS patients and to thrombotic (patients with inherited thrombophilia) and nonthrombotic controls of similar age and sex [22].

With regards to traditional cardiovascular risk factors for atherosclerosis, obesity, hypertension [20], and elevated von Willebrand factor [21] were more frequently present in primary APS but unrelated to IMT. Conversely, we identified elevated plasma homocysteine and IgG anticardiolipin (aCL) titer as independent predictors of IMT in our population [23]. A recent study also identified IgG aCL as an independent predictor of plaques in APS, but the result should be considered invalid because primary and SLE-related APS were pooled together in the analysis [24].

Peripheral vascular disease and antiphospholipid antibodies

Before APS became a distinct entity, the observation that some cases of vascular occlusion in APS were due to a proliferative vasculopathy rather than acute thrombosis [25, 26] lead to the search for an association between arterial disease and aPL. Some studies focussed essentially on aCL antibodies, others on the lupus anticoagulant. A cross-sectional survey on 234 patients undergoing vascular surgery procedures for peripheral arterial disease revealed a positive aPL test in 60 patients (26%). These aPL patients were 1.8 times more likely to have had previous lower limb vascular surgery, 5.6 times more likely to have had occlusion of that procedure, and four times more likely to be female [27]. A case-control survey on patients younger than 50 years of age operated on for atherosclerotic peripheral vascular disease revealed IgG aCL antibodies in 14.5% of the patients, mostly female. Interestingly, patients with IgG aCL had a lower prevalence of hyperlipidemia/dyslipidemia when compared to patients without these antibodies, reinforcing an atherogenic role for aCL [28]. A high prevalence of IgG aCL was also found in patients with both cerebral and peripheral artery disease from a cohort of 123 vascular disease patients (isolated cerebrovascular disease $n=18$, peripheral artery disease only $n=35$, cerebral and peripheral artery disease $n=35$, and control patients $n=35$) [29].

A further case-control study searched for the presence of the lupus anticoagulant (dilute Russell's viper venom time [DRVVT]) in surgical vascular patients (claudication $n=20$, critical ischemia $n=20$, and elective abdominal aortic aneurysm surgery $n=20$) compared to 20 general surgical controls. The platelet neutralization procedure in the DRVVT was found in 26 out of the 60 (43%) vascular patients compared with none of the 20 general surgical controls [30], though a later survey found a lower 4% prevalence of LA in 150 patients with peripheral vascular disease [31].

Aspects of atherogenesis in primary and SLE-related APS

This brief overview shows that traditional cardiovascular risk factors and disease status relate to IMT and/or plaque prevalence in SLE, but fails to identify aPL as a significant risk factor. On the other hand, IgG aCL appears as an independent predictor of IMT in primary APS where traditional risk factors do not seem to be contributory: this may account for the higher plaque prevalence in SLE than primary APS (Tables 1 and 2). However, plasma homocysteine emerged as the one risk factor that independently

Table 2 Intima–media thickness of common carotid artery in primary APS studies

	PAPS	THR-CTR	CTR	<i>p</i> value	References
No.	25		40		Jimenez et al. [9]
Age	40.2±11.4		42.2±11.2		
IMT	0.55±0.11		0.54±0.14	NS	
Plaque (%)	8		15	NS	
No.	45	40	–		Bilora et al. [19]
Age	47±13	47.2±16			
IMT	Not given	Not given			
Plaque (%)	55	75		0.07	
No.	28		28		Medina et al. [20]
Age	40±8.5		41±6.3		
IMT	2.6±1.1		1.2±0.44	0.0001	
Plaque (%)	0		3.5	NS	
No.	14 [44]	–	36		Der et al. [21]
Age	52±15		50±11		
IMT	0.714±0.2		0.58±0.08	0.003	
Plaque (%)	Unclear ^a		0		
No.	16	11	15		Margarita et al. [22]
Age (>40)					
IMT	0.588±0.119	0.568±0.080	0.495±0.074	0.03	
Plaque (%)	12	9	6		

PAPS primary antiphospholipid syndrome, THR thrombosis, CTR control, IMT intima–media thickness

^a The authors report plaques in the carotid artery of 23 patients (but the study described 44 patients in the text and 14 in their Table 1)

predicted IMT in both SLE and primary APS. Elevated plasma homocysteine shows a weak relationship to IMT in population-based studies, but the association seems stronger in subjects characterized by oxidant stress such as diabetes and chronic renal failure [32]. Homocysteine relates to enhanced lipid peroxidation in vivo [33] that in turn has an impact on blood pressure [34].

Oxidant/anti-oxidant balance in PAPS: the role of paraoxonase

Paraoxonase is an enzyme present in the arterial wall and in the HDL particle. The *PON* gene family, located in chromosome 7, codes for three types of PON whereas polymorphisms at positions 55 (methionine/leucine) and 192 (arginine/glutamine) code for PON with different enzymatic activities (reviewed in [35]). PON 1 inhibits LDL oxidation and is the major contributor to the antioxidant defense of plasma. Reduced PONa may contribute to lipid peroxidation, the process whereby LDL and other lipid-containing molecules oxidize in the bloodstream releasing [36], amongst others, vasoactive isoprostanes considered sensitive markers of in vivo oxidative stress [37]. Lipid peroxidation is implicated in atheroscle-

rosis [38] and specific by-products of lipid peroxidation promote the dissociation of the cytoplasmic nuclear factor kappa beta system [39]. The active subunit of the latter migrates to the nucleus, binds to recognition sites in the promoters of genes for vascular adhesion molecules and monocyte chemoattractant protein shifting the endothelium toward a pro-adhesive and prothrombotic phenotype. Primary and SLE-related APS are characterized by reduced PONa and enhanced lipid peroxidation [40]. The former and the latter were, respectively, inversely and positively correlated to aPL titers and both probably contribute to the atherothrombotic tendency of primary APS and possibly SLE as overgeneration of isoprostanes is linked to platelet activation [41].

Low-density lipoprotein oxidation and binding to β -2-glycoprotein I

LDL is the most proatherogenic lipoprotein but has this property only when oxidized [42, 43]. LDL contains phospholipids, free cholesterol, cholesteryl esters, triglycerides, and apolipoprotein B (apoB) that are all susceptible to oxidation [43]. As a result, polyunsaturated fatty acids broken down into a wide array of smaller fragments may

conjugate to amino lipids or to apoB [44]. In vitro studies have demonstrated a specific interaction between copper-induced LDL oxidation (Cu-oxLDL) and β_2 -glycoprotein I (β_2 GPI) [45–47]. Two chloroform-extractable lipids were identified as the LDL-derived ligands for the specific binding to β_2 GPI. These were identified as oxLig-1 and oxLig-2 (7-ketocholesteryl-9-carboxynonanoate [9-oxo-9-(7-ketocholest-5-en-3-yloxy) nonanoic acid] and 7-ketocholesteryl-1 2-carboxy (keto) dodecanoate), respectively) [47]. The in vitro interaction of Cu-oxLDL with lysine residues on β_2 GPI is initially electrostatic and reversible but later progresses to a stable bond involving a Schiff base formation. In this process, β_2 GPI neutralizes the negative charges generated by Cu-oxLDL assuming a possible antioxidant and anti-inflammatory effect alongside its anticoagulant and anti-apoptotic properties [48, 49]. Since oxLDL stimulates the release of several soluble inflammatory and adhesion molecules, induces a prothrombotic endothelial surface, and promotes inflammation attracting monocytes and T lymphocytes to the arterial intima, the possibility that β_2 GPI might blunt all these activities is highly attractive. On the other hand, given the strong immunogenic properties of oxLDL [50], it is possible that auto-antibodies against β_2 GPI-oxLDL may hamper these positive effects of β_2 GPI. From the clinical standpoint, IgG anti- β_2 GPI-oxLig1 independently predicted intima-media thickness of different carotid segments and was inversely related to decreased PONA, highlighting an important role for PON in atherogenesis related to primary APS [51].

Monocytes/macrophages

Monocytes/macrophages have receptors for LDL [52, 53] that should prevent lipid overloading. Native LDL is taken up by specific LDL receptor whereas oxLDL is taken up by scavenger receptors [54, 55]. The presence of β_2 GPI and IgG anti- β_2 GPI auto-antibodies increases the macrophage uptake of I^{131} -Cu-oxLDL in keeping with a proatherogenic role for IgG anti- β_2 GPI antibodies [46]. The uptake of the above complex is likely mediated by macrophage Fc γ receptors rather than by scavenger receptors. During uptake, circulating monocytes adhere to endothelial cells and migrate into the intima of the arterial wall where they contribute to the intima-media thickening.

HDL: a further role in APS-related atherosclerosis

HDL protects against atherosclerosis by inhibiting LDL oxidation by way of its PON content. Apolipoprotein A-I (ApoA-I), the major protein component of HDL, stabilizes the molecule and protects humans from cholesterol accu-

mulation in tissues [56]. ApoA-I retains almost identical information as HDL in terms of risk prediction for future cardiovascular disease [57]. Any interference with HDL components could compromise the protective role of HDL. In autoimmune diseases, HDL may represent a possible target for autoreactivity. Elevated levels of anti-HDL have been reported in SLE and primary APS in association with decreased PONA [41]. More recently, IgG anti-HDL and IgG anti-ApoA-I were found in association with endothelial activation markers and disease activity in SLE [58].

Unsolved issues

Some emerging issues relate complement activation to coagulation activation [59]. Complement activation is linked to several clinical features in PAPS but not to vascular disease as yet [60], though a strong association exists with tissue factor generation and pregnancy morbidity [61]. The possibility of primary APS as a low-grade inflammatory disorder has been recently confirmed [62]. The difference in plaque prevalence between SLE and primary APS is intriguing: that different levels of background inflammation might account for the difference is a simplistic view, but endothelial cell apoptosis, plaque formation, and subsequent fibrosis may represent a repair mechanism in an undesirable site: plasma levels of a fibrogenic cytokine (transforming growth factor beta) is increased in SLE [63].

Management of aPL-related atherosclerosis

At present, we do not know how to manage SLE- and APS-related atherosclerosis. Control of modifiable risk factors is intuitive. Treatment may be directed at managing the effects of aPL antibodies or at decreasing aPL titers. Given their positive effects on the immune system, on the vascular endothelium, and on PONA, statins may be suitable candidates within the context of a clinical trial. Probucol showed some positive effects on atherogenic pathways in primary APS [64, 65]. Anti-CD20 accomplished lowering of aPL titers in some primary APS patients but not others [66–68].

Concluding remarks

Despite the early excitement for aPL as a risk factor for atherosclerosis in SLE, this brief overview does not support aPL as risk factor for IMT and plaque development and progression in SLE. The data are slightly more convincing in primary APS but further studies are required

on patients well-stratified in definite age groups rather than spanning across different age groups. Suitable controls should not be only healthy individuals but also subjects with inherited thrombophilia to match the persistent exposure to a thrombotic risk factor and the use of oral anticoagulation.

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