The association of antiphospholipid antibodies with cardiopulmonary manifestations of systemic sclerosis

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ABSTRACT

Objective. *To determine the prevalence and correlates of antiphospholipid antibodies (APLA) in systemic sclerosis (SSc).*

Methods. *Nine hundred and forty SSc patients were tested for APLA using an ELISA assay at recruitment. Clinical manifestations were defined as present, if ever present from SSc diagnosis. Logistic regression analysis was used to determine the associations of APLA.*

Results. *One or more types of APLA were present in 226 (24.0%) patients. Anticardiolipin (ACA) IgG (ACA-IgG) antibodies were associated with right heart catheter-diagnosed pulmonary arterial hypertension (PAH), with higher titres corresponding with a higher likelihood of PAH (moderate titre (20–39 U/ml) ACA-IgG odds ratio [OR] 1.70, 95% CI: 1.01–2.93, p=0.047; high titre (>40 U/ml) ACA-IgG OR 4.60, 95% CI:1.02–20.8, p=0.047). Both ACA-IgM (OR 2.04, 95% CI: 1.4–3.0, p<0.0001) and ACA-IgG (OR 1.84, 95% CI: 1.2–2.8, p=0.005) were associated with interstitial lung disease (ILD). Increasing ACA-IgM and IgG titres were associated with increased likelihood of ILD. ACA-IgG was a marker of coexistent pulmonary hypertension and ILD (ILD-PH) (OR 2.10, 95% CI: 1.1–4.2, p=0.036). We also found an association between ACA-IgG and digital ulcers (OR 1.76, 95% CI: 1.16–2.67, p=0.008) and ACA-IgM and Raynaud's phenomenon (OR 2.39, 95% CI: 1.08-5.27, p=0.031). There was no association between APLA and SSc disease subtype, peak skin score, presence of other autoantibodies, mortality or other disease manifestations.*

Conclusion. *The association of APLA with PAH, ILD, ILD-PH, Raynaud's phenomenon and digital ulcers suggests that endothelial abnormalities and small vessel thrombosis may be important in the pathogenesis of these disease features.*

Introduction

Systemic sclerosis (SSc) is a potentially devastating connective tissue disease. It affects women up to seven times as often as men and frequently during mid to late child bearing years (1). It is characterised by three histopathological features: peri-vascular and tissue infiltration of mononuclear inflammatory cells, functional and structural vascular lesions and increased synthesis and deposition of collagen within skin and internal organs (2). SSc is thought to be an autoimmune disease, and autoantibodies against a variety of extractable nuclear antigens can be detected in patient sera (3). The pathogenesis of SSc remains unknown, although endothelial dysfunction has been suggested as a key element in the disease process.

Antiphospholipid antibodies (APLA) are a heterogeneous group of circulating immunoglobulins present in the antiphospholipid syndrome and a wide range of other autoimmune conditions such as systemic lupus erythematosus (SLE) (4-6). The most commonly detected subgroups include anticardiolipin antibodies (ACA), lupus anticoagulant (LA) and antibodies to beta-2-glycoprotein (anti-β2GP). Typically, their presence is correlated with arterial and venous thrombosis, livedo reticularis, recurrent foetal loss, thrombocytopenia, and cerebral and myocardial infarction (4, 6).

The mechanism of APLA-induced thrombosis is not fully understood. Multiple mechanisms have been proposed and recently summarised by Giannakopouls and Krilis (7). The proposed mechanisms include increased oxidative stress, impaired function of

endothelial nitric oxide-dependent vascular relaxation, activation of receptors by anti-B2GP1 antibodies, increased expression and activation of tissue factor, increase in the free thiol form of factor X1, disruption of the annexin A5 shield and antibody-mediated activation of complement C3 and C5 (6, 7).

A role for APLA in the pathogenesis of SSc has been postulated (2). However, little is known about the clinical associations of these antibodies in this disease. Further, the studies performed to date have been of small patient numbers (<120 patients).

We sought to determine the prevalence and clinical manifestations of APLA in the largest cohort of patient with SSc yet studied for this objective.

Materials and methods

Patients

All patients were recruited from the Australian Scleroderma Cohort Study (ASCS). As previously described (8), this is a prospective cohort of over 1200 Australian SSc patients who have undergone at least one detailed clinical assessment, along with screening for cardiopulmonary complications since 2007, using annual echocardiography and pulmonary function tests, and high-resolution computerised tomography (HRCT) lung, where indicated. Patients were attending one of 12 ASCS screening centres and gave informed consent at recruitment. The study was approved by the Human Research Ethics Committees of each participating institution.

Methods

All enrolled patients participating in the ASCS who fulfilled the American College of Rheumatology (ACR) or Leroy/Medsger criteria (9, 10) for SSc were tested for APLA, specifically ACA-IgM, ACA-IgG and anti-β2GP, using commercial ELISA assays (cardiolipin Vital Diagnostics assay and Orgentec beta2 glycoprotein assay) at the initial visit. Low titre was defined as <20 U/mL, moderate titres as 20–39 U/mL and high titre as >40 U/mL. Lupus Anticoagulant was measured by a three step strategy including an activated partial thromboplastin time (APTT) screening test (demonstration of prolongation of a phospholipid dependent clotting time beyond the upper limit of the reference interval); a dilute Russell's viper venom time mixing test (confirmation of the presence of an inhibitor and exclusion of a coagulation factor deficiency by combining the patient's plasma with normal plasma and assessing its influence on clotting time) and finally confirmation that the inhibitor is phospholipid dependent and not directed against a specific coagulation factor.

Antiphospholipid antibodies (APLA), other autoantibodies (measured using commercial ELISA assays), and various clinical manifestations were defined as present, if ever present from SSc diagnosis. Disease subtype was classified based on the extent of skin involvement, with limited disease being confined to extremities and face. Modified Rodnan skin score (mRSS) was used to measure skin involvement (11, 12). Its scores range from 0 to 51, with higher scores indicating more severe skin involvement.

Definite pulmonary arterial hypertension (PAH) was defined as per current Dana Point criteria (13) with right-heart catheter (RHC) determined mean pulmonary arterial pressure (mPAP) >25mmHg and pulmonary capillary wedge pressure (PCWP) ≤15mmHg. Importantly, none of the patients in this study had chronic thromboembolic pulmonary hypertension (CTEPH), based on the treating physician assessment together with ventilation perfusion scanning and CT pulmonary angiography where appropriate. Interstitial lung disease (ILD) without definite PAH was defined as moderate or severe ILD on CT with a forced vital capacity (FVC) <85%, and RHC showing a mPAP <25mmHg, or where this was unavailable, a transthoracic echocardiography systolic pulmonary artery pressure (TTE sPAP) <40mmHg. ILD with pulmonary hypertension (ILD-PH) was defined as significant ILD and a RHC showing mPAP >25mmHg and PCWP ≤15mmHg. Renal crisis was defined as the abrupt onset of severe hypertension (systolic blood pressure (BP) ≥180 mmHg and/or diastolic BP ≥100 mmHg) without an alternate aetiology, with or without microangiopathic anaemia, and / or decline in renal function without an alternate aetiology. Gastrointestinal involvement was defined as one or more of reflux oesophagitis, oesophageal stricture or dysmotility, gastric antral vascular ectasia, bowel dysmotility or pseudo-obstruction. Myocardial involvement was defined as the presence of either left ventricular systolic or diastolic dysfunction where no other cause was identified, or a conduction disturbance unexplained by other mechanisms, or a characteristic histological picture on endomyocardial biopsy.

Statistical analysis

All descriptive data are presented as n (percentage) or mean (standard deviation; SD). Univariate methods (Chi-square and Fisher's exact tests) were used to identify the clinical associations of APLA. Univariate logistic regression analysis was used to quantify these associations, which were then adjusted for age and disease duration in multiple regression analysis. All analyses were performed using STATA 11.0 (Statacorp, College Station, TX, USA).

Results

Patient and disease characteristics

The patients (n=940) were predominantly female (87.7%) with limited disease subtype (63.5%), mean (SD) age at enrolment of 57.5 (12.5) years, and mean (SD) disease duration at the time of study of 15.3 (13.9) years (Table I). The clinical manifestations and their prevalence were Raynaud's phenomenon (89.7%), digital ulcers (45%), ILD (26.8%), PAH (13.2%), ILD-PH (5.3%), renal crisis (4%), myocardial disease (7.7%) and gastrointestinal involvement (100%). Sixty-two (6.6%) patients had ever had deep vein thrombosis (DVT), while 29 (3.1%) had ever had pulmonary emboli (PE). Fifty (5.3%) patients died during the follow-up period of 2.14 (0.88) years.

Frequency of APLA

One or more APLA were found in 24% of patients (Table II). In most cases the antibody titres were low. ACA-IgM had the highest prevalence at 14.1% followed by ACA-IgG at 10.3% and anti-β2GP at 6.7%. No patients were positive for lupus anticoagulant.

Associations of APLA

The associations of APLA with disease manifestations are presented in Tables III and IV. As there was no change after adjustment for age and disease duration, we have presented only the univariate associations. Positive ACA-IgG and high titre ACA-IgG were associated with a 1.7- and 4.6-fold increased risk of PAH, respectively, with higher titres corresponding with a higher risk of PAH (ACA-IgG odds ratio [OR] 1.70, 95% CI: 1.01–2.93, p=0.047 and high titre ACA-IgG OR 4.60, 95% CI: 1.02–20.8, p=0.047, respectively). ACA-IgM and ACA-IgG were associated with 2.04 and 1.84-fold increased risk of ILD, respectively, with higher titres associated with higher risk (moderate titre ACA-IgM OR 2.36, 95% CI: 1.17–4.76, p=0.016, and moderate titre ACA-IgG OR 2.15, 95% CI: 1.03–4.50, p=0.041). Anticardiolipin IgG (ACA-IgG) was associated with a 2-fold increased risk of ILD-PH (OR 2.10, 95% CI: 1.05–4.2, p=0.036) and a 1.76-fold increased risk of digital ulcers (OR 1.76, 95% CI: 1.16–2.67, p=0.008), while ACA-IgM were associated with 2.39-fold increased risk of Raynaud's phenomenon (OR 2.39, 95% CI: 1.08–5.27, p=0.031).

Negative associations

There was no association (p>0.1) between APLA and peak modified Rodnan skin score (in the highest quartile, which was >16). Similarly, we did not find an association between APLA and PAH severity (defined as the highest quartile of values for mPAP on RHC (>40mmHg)). There was no significant association between APLA and other disease manifestations such as myocardial disease, various gastrointestinal manifestations and renal crisis, nor between APLA and DVT or PE in this group of patients. Further, there was no association between APLA and all-cause mortality, or mortality due to PAH or ILD or ILD-PH.

Table I. Patient characteristics (n=940).

Characteristic	n (%) or mean (SD)
Total number of patients	940 (100%)
Sex	
Female	824 (87.7%)
Male	116 (12.3%)
Disease subtype (diffuse *vs*. limited)*	
Limited	597 (63.5%)
Diffuse	243 (25.9%)
Disease duration at entry (years)	15.3 (13.9)
Age at entry (years)	57.5 (12.5)
Autoantibodies	
ANA	871 (93.0%)
Anti-Scl70	134 / 918 (14.6%)
Anti-centromere	395 / 921 (42.9%)
Anti-RNAP	68 (13.8%)
Clinical manifestations	
Digital ulcers	423 (45.0%)
Raynaud's	843 (89.7%)
ILD[#]	252 (26.8%)
PAH[¶]	124 (13.2%)
Coexistent ILD-PH[§]	50 (5.3%)
Renal crisis[ᵅ]	38 (4.0%)
Myocardial disease[†]	72 (7.7%)
Gastrointestinal involvement[‡]	940 (100%)
Deep vein thrombosis	62 (6.6%)
Pulmonary emboli	29 (3.1%)
Died during follow-up	50 (5.3%)

ANA: antinuclear antibody; Anti-Scl 70: anti-topoisomerase antibodies; ACA: anti-centromere antibody; Anti-RNAP: anti-RNA polymerase III antibody.
Autoantibodies were measured at recruitment and disease manifestations are defined as present ever from diagnosis.
*Disease subtype classified based extent of skin involvement, with limited disease being confined to extremities and face.
[#]Interstitial lung disease without definite PAH was defined as moderate or severe ILD on CT with a forced vital capacity (FVC) <85%, and RHC showing a mPAP <25mmHg, or where this was unavailable, a TTE sPAP<40mmHg.
[¶]Pulmonary arterial hypertension defined as mean pulmonary arterial pressure ≥ 25 mmHg at rest with pulmonary capillary wedge pressure ≤15 mmHg.
[§]Coexistent ILD-PH defined as moderate or severe ILD on CT and a RHC showing a mPAP >25mmHg and PCWP ≤15mmHg.
[ᵅ]Renal Crisis defined as abrupt onset severe hypertension (systolic blood pressure (BP) ≥180 mmHg and/or diastolic BP ≥100 mmHg) without an alternate etiology, with or without rising creatinine or microangiopathic anaemia.
[†]Myocardial disease defined as the presence of either left ventricular systolic or diastolic dysfunction where no other cause was identified, or a conduction disturbance unexplained by other mechanisms, or a characteristic histological picture on endomyocardial biopsy.
[‡]Gastrointestinal involvement defined as one or more of reflux oesophagitis, oesophageal stricture or dysmotility, gastric antral vascular ectasia, bowel dysmotility or pseudo-obstruction.

Discussion

The prevalence of APLA in SSc within our study was 24%, with the majority being ACA. This is consistent with existing literature. Although ranging widely (0 – 63%), most studies report an overall prevalence of 20-25% (14), the majority being ACA (2). Previous studies have reported a low prevalence, 4%, positivity for LAC while none of our patients had LAC (2, 14).

With the largest sample size to date of 940 patients, we were able to evaluate the clinical correlates of APLA. We have shown an association between ACA-IgG and PAH, ILD-PH and digital ulcers, an association between ACA-IgM and Raynaud's phenomenon, and an association between ACA-IgM and IgG and ILD. We found no association between β2GP antibodies and PAH or ILD. In contrast with previous studies, we did not show an association with other disease manifestations. The difference between our results and those of previously published data may be related to the smaller sample sizes of other studies.

Our study found no significant association between APLA and disease subtype, as previously reported (2, 15) although other studies have found a correlation between ACA-IgG and more severe disease defined by higher skin score (2, 5, 14, 16). The mechanism of this relationship is not clear (2, 5, 16),

Table II. Type and titre of antiphospholipid antibodies (n=226).

Antibody titre U/ml	Antibody type n (%)		
	ACA IgM	ACA IgG	Anti-B_2GP
<20	100 (10.6%)	68 (7.2%)	44 (4.7%)
20 to 39	20 (2.1%)	23 (2.4%)	9 (0.9%)
≥40	13 (1.4%)	7 (0.7%)	10 (1.1%)

ACA: anticardiolipin antibody; Anti-β2GP: antibodies to beta-2-glycoprotein.

Table III. Univariate comparison of correlates of APLA.

Disease manifestation**	ACA type	n (% of patients with antibody)	n (% of patients without antibody)	*p*-value
PAH[¶] (n=124)	ACA-IgG	20/95 (21.05%)	104/774 (13.4%)	0.045[ᵡ]
	ACA-IgG >40	3/7 (42.9%)	121/864 (14.0%)	0.06[§]
ILD[#] (n=226)	ACA-IgM	52/129 (40.31%)	171/727 (23.52%)	<0.0001[ᵡ]
	ACA-IgM >20	14/30 (46.67%)	212/841 (25.21%)	0.008[ᵡ]
	ACA-IgG	36/95 (37.89%)	190/774 (24.55%)	0.005[ᵡ]
	ACA-IgG >20	13/29 (44.83%)	213/842 (25.3%)	0.018[ᵡ]
ILD-PH[§] (n=50)	ACA-IgG	10/95 (10.53%)	40/774 (5.17%)	0.034[ᵡ]
Digital ulcers (n=386)	ACA-IgG	53/95 (55.79%)	333/774 (43.02%)	0.018[ᵡ]
Raynaud's (n=778)	ACA-IgM	91/95 (95.79%)	687/774 (88.76%)	0.035[ᵡ]

ACA: anticardiolipin antibody; ILD: interstitial lung disease; ILD-PH: interstitial lung disease-related pulmonary hypertension; PAH: pulmonary arterial hypertension.
[¶]Pulmonary arterial hypertension defined as mean pulmonary arterial pressure ≥25 mmHg at rest with pulmonary capillary wedge pressure ≤15 mmHg.
[#]Interstitial lung disease without definite PAH was defined as moderate or severe ILD on CT with a forced vital capacity (FVC) <85%, and RHC showing a mPAP <25mmHg, or where this was unavailable, a TTE sPAP<40mmHg.
[§]ILD-PH defined as moderate to severe ILD on CT and a RHC showing a mPAP >25mmHg and PCWP ≤15mmHg.
**present ever from diagnosis; [ᵡ]Chi-square test; [§]Fisher's exact test.

and in our study, there was no association between APLA and skin score.

An association of APLA with the vascular manifestations of SSc has been variably reported. For example, Boin *et al*. showed an association between anti-β2GP and digital loss, active digital ischaemia and higher mortality (17). Picillo *et al*. demonstrated a correlation between ACA-IgG and myocardial ischaemia or necrosis (16). Marie *et al*. reported an association between APLA and peripheral ischaemia of the lower limbs (2). Wielosz *et al*. identified an association between ACA-IgG and anti-β2GP IgG and proteinuria, reduced glomerular filtration and renal crisis (5). We found an association between APLA and digital ulcers and Raynaud's phenomenon but there was no significant association between APLA and other microvascular manifestations, cardiac involvement, peripheral vascular disease or renal involvement, specifically renal crisis. We also found no association between APLA and other autoantibodies found in SSc.

PAH is a devastating vascular complication of SSc, with a significant impact on quality of life, and together with ILD is the most common cause of SSc-related death (1, 18-22). The proposed aetiology of PAH in SSc (SSc-PAH) involves endothelial cell proliferation, pulmonary vascular disease from increased muscularisation of vessel walls with deposition of immunoglobulin G (IgG) in and around occluded vessels, and intrapulmonary small vessel thrombosis (6, 19). These mechanisms mirror the putative actions of APLA. Our study indicated that moderate to high titres of ACA-IgG were associated with a 2-fold increased risk of isolated PAH, diagnosed by RHC, as has been previously documented (2, 15). We found a gradient of association between APLA titre and the odds of ever developing PAH. Specifically, a positive ACA-IgG was associated with a 1.7-fold increased likelihood of developing PAH, while high titre ACA-IgG was associated with a 4.6-fold increased likelihood of PAH. On the other hand, we did not find an association with increasing mPAP on RHC, although this has been previously reported by Ihn *et al*. (15). We found that moderate to high titres of ACA-IgG were associated with a 2-fold increased risk of ILD-PH. In addition, moderate to high titres of ACA-IgM and IgG were associated with a 2-fold increased risk of ILD, with higher APLA titre corresponding with higher likelihood of developing ILD. This contrasts with the findings of Marie *et al*., who found no significant difference in APLA status among those who had ILD and those who did not (2). A possible explanation for the association of APLA with ILD is an immune-based microvascular injury syndrome (20), which has been shown pathologically by Magro *et al*. (23), indicating that the role of the pulmonary vasculature in the development and progression of ILD may have been underestimated.

A limitation of our study was that we were unable to evaluate the role of APLA in pregnancy and foetal loss as the mean age at disease onset in our study was 57.5 years, meaning that most patients had already finished child bearing at the time of disease onset. Although the association between ACA and anti-β2GP, and DVT and livedo reticularis is well-known (24), our study showed no association between APLA and DVT or PE. We also found no association between β2GP antibodies and PAH or ILD. This may be because there are fewer patients with scleroderma that have β2GP antibodies, and we may have been underpowered to show an association. Another limitation of the study is that APLA were measured only at baseline and we were unable to quantify the change in APLA titres over time or evaluate the relationship between changes in APLA levels and SSc disease manifestations. Finally, as this was a prevalent rather than incident cohort, many disease manifestations defined as 'present ever' would have been recorded, retrospectively.

The common association of Raynaud's, digital ulcers and PAH with APLA suggest that these vascular manifestations

Table IV. Correlates of APLA determined using logistic regression.

Disease manifestation**	ACA type	Odds ratio	95% CI	*p*-value
PAH[¶]	ACA-IgG	1.70	1.01-2.93	0.047
	ACA-IgG >40	4.60	1.02-20.8	0.047
ILD[#]	ACA-IgM	2.04	1.40-3.00	<0.0001
	ACA-IgM >20	2.36	1.17-4.76	0.016
	ACA-IgG	1.84	1.20-2.83	0.005
	ACA-IgG >20	2.15	1.03-4.50	0.041
ILD-PH[§]	ACA-IgG	2.10	1.05-4.20	0.036
Digital ulcers	ACA-IgG	1.76	1.16-2.67	0.008
Raynaud's	ACA-IgM	2.39	1.08-5.27	0.031

ACA: anticardiolipin antibody; CI: confidence interval; ILD: interstitial lung disease; ILD-PH: interstitial lung disease-related pulmonary hypertension; MRSS: Modified Rodnan Skin Score; PAH: pulmonary arterial hypertension.
[¶]Pulmonary arterial hypertension defined as mean pulmonary arterial pressure ≥25 mmHg at rest with pulmonary capillary wedge pressure ≤ 15 mmHg.
[#]Interstitial lung disease without definite PAH was defined as moderate or severe ILD on CT with a forced vital capacity (FVC) <85%, and RHC showing a mPAP <25mmHg, or where this was unavailable, a TTE sPAP<40mmHg.
[§]ILD-PH defined as moderate to severe ILD on CT and a RHC showing a mPAP >25mmHg and PCWP ≤15mmHg.
*Disease subtype classified based extent of skin involvement, with limited disease being confined to extremities and face. **present ever from diagnosis.

may have similar pathogenic mechanisms (25). The association between APLA and PAH supports the role of micro-thrombosis in the pathophysiology of PAH and may be an explanation for the survival advantage seen with anticoagulation in some observational studies (26, 27). Possible applications of our findings include the use of APLA to identify patients at high risk of developing PAH and a means for gaining better insight into disease pathogenesis so that targeted therapies may be developed.

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