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Prévalence et associations cliniques des anticorps anti-phospholipides dans la sclérodermie systémique : données d'une cohorte lilloise et revue systématique de la littérature

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Liste des abréviations

ACA	Anti-centromere antibody
ACR	American College of Rheumatology
aCL	Anti-cardiolipin antibody
anti-β2GpI	Anti-β2 glycoprotein-I antibody
anti-RNAPIII	Anti-RNA polymerase III antibody
anti-topo I	Anti-topoisomerase I antibody
aPL	Anticorps anti-phospholipides, antiphospholipid antibodies
APS	Antiphospholipid syndrome
AT	Arterial thrombosis
dSSc	Diffuse cutaneous systemic sclerosis
DU	Digital ulcer
DVT	Deep venous thrombosis
EULAR	European League Against Rheumatism
HTAP	Hypertension artérielle pulmonaire
IC 95	Intervalle de confiance de 95%
IgG	Immunoglobulin G
ILD	Interstitial lung disease
LA	Lupus anticoagulant
lcSSc	Limited cutaneous systemic sclerosis
PAH	Pulmonary arterial hypertension
PAPS	Primary antiphospholipid syndrome
PE	Pulmonary embolism
SAPL	Syndrome des anti-phospholipides
SAPS	Secondary antiphospholipid syndrome
ScS	Sclérodermie systémique
SSc	Systemic sclerosis
VT	Venous thrombosis
95% CI	95% confidence interval

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RESUME

Contexte. Des anticorps anti-phospholipides (aPL) peuvent être retrouvés dans le sérum des patients sclérodermiques. Des variations importantes de leur prévalence sont observées dans la littérature. Leurs associations cliniques restent largement méconnues. Cette étude avait pour objectifs de déterminer la prévalence des aPL dans une cohorte de patients sclérodermiques, d'évaluer leurs associations cliniques et de réaliser une revue systématique de la littérature.

Méthode. Dans notre centre, une recherche d'anticoagulant lupique, d'anticorps anti-cardiolipine (aCL) et d'anticorps anti-β2glycoproteine I (anti-β2GpI) a été réalisée chez 249 patients sclérodermiques de manière consécutive. Les associations cliniques ont été étudiées. Une revue systématique de la littérature a été réalisée via les bases Embase et PubMed.

Résultats. Au moins un anticorps était présent chez 16 patients (6.4% IC95 [3.4-9.5]). La présence d'aPL était associée aux thromboses veineuses (TV) en analyse univariée (OR = 3.91; IC95 [0.98-13.53]; p=0.027), et une tendance statistique était retrouvée en analyse multivariée (OR 3.24; IC95 [0.87-10.9]; p=0.064). En analyse multivariée, la présence d'aPL était associée aux pertes fœtales (OR 4.31; IC95 [1.09-16.33]; p=0.031), les titres élevés d'aCL (≥5 UGPL/mL) étaient associés à l'hypertension artérielle pulmonaire (HTAP) (OR 6.35; IC95 [1-41.1]; p=0.043) et aux thromboses veineuses (OR 3.69; IC95 [0.98-12.9]; p=0.043). Les titres élevés d'anti-β2GpI et les pertes fœtales étaient associés (OR 5.25 ; IC95 [1.04-27.1]; p=0.041). Vingt-quatre études ont été sélectionnées suite à la revue systématique de la littérature, représentant une population de 2992 patients sclérodermiques. La

prévalence des aPL dans cette population était très variable (entre 0 et 58%). La manifestation clinique associée à la présence d'aPL la plus fréquente était l'HTAP.

Conclusion. Cette étude retrouve une prévalence des aPL dans la ScS de 6.4% (IC95 [3.4-9.5]). La présence d'aPL était associée aux TV et aux pertes fœtales. Ces données apportent des éléments supplémentaires concernant l'atteinte vasculaire de la ScS.

INTRODUCTION GENERALE

La sclérodermie systémique (ScS) est une maladie auto-immune, de type connectivite. Elle est caractérisée par une atteinte vasculaire, une atteinte fibrosante et la présence d'une auto-immunité. Comme dans les autres connectivites les manifestations cliniques sont variées : sclérose cutanée plus ou moins étendue, reflux gastro-œsophagien, phénomène de Raynaud, ulcères digitaux, pneumopathie interstitielle, hypertension artérielle pulmonaire (HTAP), calcinose, arthrite, myosite. Ces atteintes d'organes ne sont pas présentes chez tous les patients, ou à des degrés divers. Certaines d'entre elles peuvent engager le pronostic vital.

Différents auto-anticorps dirigés contre des composants cellulaires ont été mis en évidence dans le sérum des patients. Le rôle pathogène des auto-anticorps dans la ScS n'est pas encore clairement identifié. Néanmoins ils se révèlent fortement associés au phénotype clinique des patients constituant de véritables biomarqueurs diagnostiques et pronostiques de la maladie utilisés en pratique quotidienne.

Des anticorps anti-phospholipides (aPL) peuvent être retrouvés dans le sérum de patients sclérodermiques. La présence des aPL est un critère indispensable d'une autre pathologie auto-immune : le syndrome des antiphospholipides (SAPL), associé à des manifestations cliniques telles que des événements thrombotiques veineux ou artériels, des événements obstétricaux (fausses couches précoces répétées, perte fœtale tardive, éclampsie). D'autres manifestations cliniques peuvent être observées comme un livedo, une thrombopénie… Le SAPL est défini comme primaire s'il est isolé, ou secondaire lorsqu'il est associé à une connectivite.

De nombreuses études ont étudié la prévalence et les associations cliniques des APL dans la ScS. Néanmoins les résultats de ces travaux sont hétérogènes et les conclusions difficiles à dessiner. Dans la plupart des cas les cohortes sont de petite taille, ce qui induit une puissance statistique faible. Certaines présentent des biais méthodologiques (population sélectionnée, seuil de positivité des tests biologiques différents). De plus, les 3 types d'anticorps habituellement décrits dans le SAPL (anticoagulant lupique, anticorps anti-cardiolipine, anticorps anti-β2glycoprotéine I) sont rarement testés simultanément.

Ce travail de thèse avait donc pour objectifs : (i) d'étudier la prévalence des aPL dans la ScS et leurs associations cliniques dans notre cohorte lilloise ; (ii) de réaliser une revue systématique de la littérature mondiale sur la prévalence des aPL dans la ScS.

ABSTRACT

Background Antiphospholipid antibodies (aPL) can be present in the sera of systemic sclerosis (SSc) patients. Important variations of their prevalence are observed in the literature. Their clinical associations remain largely unknown. This study aimed to determine the prevalence of aPL in a cohort of SSc patients, to assess their clinical associations and to perform a systematic review of the literature.

Method In our center, 249 SSc patients were consecutively tested for lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2glycoproteine I (anti-β2GpI) antibodies. Clinical associations were studied. A systematic review of the literature was carried out in PubMed and Embase.

Results One or more type of aPL was present in 16 patients (6.4%; 95%CI [3.4-9.5]). aPL positivity was associated with venous thrombosis (VT) in univariate analysis (OR = 3.91; 95%CI [0.98-13.53]; p=0.027); there was a trend towards an association in multivariate analysis (OR 3.24; 95%CI [0.87-10.9]; p=0.064). aPL positivity was associated with miscarriage in multivariate analysis (OR 4.31; 95%CI [1.09-16.33]; p=0.031). In multivariate analysis, high titers of aCL (>5 UGPL/mL) were associated with PAH (OR 6.35; 95%CI [1-41.1]; p=0.043) and venous thrombosis (OR 3.69; 95%CI [0.98-12.9]; p=0.043). High titers of anti-β2GpI and miscarriage were associated (OR 5.25; 95%CI [1.04-27.1]; p=0.041).

Twenty-four studies were retrieved by the systematic review, representing a total population of 2992 SSc patients. The prevalence of aPL in this population was highly

variable (range 0-58%). Clinical manifestations associated with aPL positivity were more frequently PAH.

Conclusion This study found a prevalence of aPL in SSc of (6.4% 95%CI [3.4-9.5]). aPL positivity was associated with VT and miscarriage. These data provide additional insights into the vascular involvement of SSc.

INTRODUCTION

Systemic sclerosis (SSc) is a chronic connective tissue disorder. Immune activation, vascular damage, and excessive synthesis of extracellular matrix with collagen deposition are all known to be important in development of this disease (1). Autoantibodies against multiple cellular components can be detected in patients' sera. The most common antinuclear auto-antibodies found in SSc are anti-centromere (ACA), anti-topoisomerase I (anti-topo I) and anti-RNA polymerase III (anti-RNAPIII) antibodies; they are used as diagnostic biomarkers. Each of these biomarkers are associated with different clinical manifestations and various degrees of SSc severity, but their contribution to pathogenesis remain unclear (2). There are some evidences that certain SSc specific autoantibodies, but also newly discovered endothelium-related antibodies, may lead to vasculopathy. Indeed, auto-antibodies are strong predictor of microvascular damage (SSc auto antibodies are associated with naifold capillary miscroscopy patterns and telangiectases) (3). An association between levels of antibodies and clinical manifestations has been described for antibodies against angiotensin II type 1 receptor and endothelin-1 type A receptor. Both autoantibodies induced extracellular signal-regulated kinase 1/2 phosphorylation and increased TGFβ gene expression in endothelial cells which could be blocked with specific receptor antagonists (4). Servettaz and al. reported that the main target of anti-endothelial cell antibodies (AECA) in patients with limited cutaneous SSc was the nuclear and ubiquitous protein CENP-B (5).

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies. Lupus anticoagulant (LA), and/or anticardiolipin antibody (of IgG and/or IgM isotype) (aCL) and/or anti-β2 glycoprotein-I antibody (of IgG and/or IgM isotype) (anti-β2GpI) are

usually detected in antiphospholipid syndrome (APS), known as primary APS (PAPS), when they are associated with thrombosis events (arterial or venous), recurrent miscarriage, fetal loss or obstetrics events; or secondary APS (SAPS), if this syndrome is associated with a connective disease (usually systemic lupus erythematosus). These antibodies are strong biomarkers for APS diagnosis and prognostic (6).

aPL have also been found in patients with a variety of other autoimmune and rheumatic diseases (7). However, in the absence of clinical events classically associated with the APS, their significance remains unclear. Moreover, their role in the development of specific organ involvement usually associated with these rheumatic diseases is unknown.

In SSc, clinical and pathologic findings of vascular damage and endothelial cell activation strongly support the hypothesis of a vascular disease as an important and primary process. This vasculopathy affects small or large vessels. The clinical manifestations of microangiopathy are Raynaud phenomenon, digital ulcers and critical digital ischemia, and renal crisis. Typical abnormalities of microangiopathy on capillaroscopy are enlarged capillaries, bushy capillary formations, microhemorrhages, and a variable loss of capillaries with or without avascular areas. Vasculopathy can also affect the pulmonary circulation leading to pulmonary arterial hypertension (8).

In the literature, there are important variations in the estimation of the prevalence of aPL in SSc. The estimated prevalence ranged from 0 to 57%. Moreover, associations of these antibodies with thrombotic events or SSc clinical manifestations are debated (9,10). Some studies reported an association between aPL positivity in SSc and pulmonary arterial hypertension (PAH) (11–13), digital ulceration (DU) (13,14), interstitial lung disease (ILD) (13), while others did not (15,16). Most of these studies

have tested a relatively small number of patients, which could be responsible for a lower statistical power. Some of them had methodological bias (selected population, different cutoff value of biological tests). These heterogeneous results preclude any further conclusion on a link between aPL positivity and clinical manifestation in SSc. The aims of this study were: (i) to determine the prevalence of aPL in a cohort of SSc patients and their clinical associations, (ii) to perform a systematic review of published reports to estimate the prevalence of aPL in SSc.

PATIENTS AND METHODS

I. Lille cohort of patients

Two hundred and forty-nine unselected patients with SSc were consecutively included and studied in the internal medicine department of University Hospital of Lille, France, between October 2014 and January 2016. Patients fulfilled the following criteria for inclusion: age>18 years, and a diagnosis of SSc according to ACR/EULAR criteria (17,18). Disease subtype was classified based on LeRoy and Medsger criteria: diffuse cutaneous SSc (dSSc) and limited cutaneous SSc (lcSSc) (19).

All variables were entered into a standardized questionnaire fulfilled by the clinician at the time of the inclusion. Collection of complete medical history, physical examination variables, laboratory and imaging exams were conducted for all patients. Interstitial lung disease (ILD) was defined as subpleural pure ground-glass opacities and/or interstitial reticular pattern with or without traction bronchiectasis and/or honeycomb cysts, on high resolution computed tomography (HRCT). Pre-capillary pulmonary hypertension was diagnosed based on right-heart catheterization if mean pulmonary arterial pressure was ≥25mmHg and pulmonary capillary wedge pressure ≤15mmHg. Pulmonary arterial hypertension (PAH) was considered in patients with pre-capillary pulmonary hypertension and no ILD or ILD with forced vital capacity % predicted ≥ 70% and extent of ILD on HRCT ≤ 20% (20). Renal crisis was defined as the abrupt onset of severe hypertension and/or decline in renal function, with proteinuria without an alternate etiology.

In accordance with French legislation, written information was provided and consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki and complied with the requirements of the French Commission Nationale Informatique et Libertés (no. DC- 2008-642).

All patients were tested for LA, aCL (IgG isotype) and anti-β2GpI (IgG isotype). LA was detected in plasma by a dilute Russell's viper venom time (Siemens), and partial thromboplastin time test (HemosIL Silica Clotting Time Werfen) as screening and confirmation tests with calculating a normalized ratio. aCL and anti-β2GpI were measured using commercial ELISA assays (Orgentec, Trappes, France), positive titer was defined as ≥ 10 UGPL/mL (aCL) or ≥ 10 UA /mL (anti-β2GpI). Other laboratories tests performed at the inclusion were: creatinine, CRP, platelet count, uric acid, serum protein electrophoresis, immunoglobulin G, M and A plasma levels, prothrombin time, activated partial thromboplastin time, lipids profile, glycated hemoglobin, rheumatoid factor, cryoglobulin, and NT-proBNP.

II. Systematic review

a. Search strategy

Three of the investigators (AL, VS and DL) performed independent searches of published studies between May 1975 and November 2015, in PubMed and Embase databases. We used the following search equation adapted to the peculiarities of each database:

(systemic sclerosis[MeSH Terms]) AND ((antibodies, antiphospholipid[MeSH Terms]) OR (antibody syndrome, antiphospholipid [MeSH Terms]) OR (antibodies,

anticardiolipin[MeSH Terms]) OR (anti phospholipid antibody syndrome[MeSH Terms]) OR (lupus anticoagulant[MeSH Terms]) OR (anti-β2GP1) OR (anti-phosphatidylethanolamine) OR (thrombosis) OR (pulmonary embolism[MeSH Terms]) OR (deep vein thrombosis[MeSH Terms]) OR (cavernous sinus thrombosis[MeSH Terms]) OR (acute stroke[MeSH Terms]) OR (stroke[MeSH Major Topic]) OR (myocardial infarct[MeSH Terms]) OR (acute anterior wall myocardial infarction[MeSH Terms]) OR (pregnancy))

After screening the titles and abstracts, studies were selected after a full-length review by two investigators (AL and VS). The complete articles were reviewed independently by the investigators, and selection criteria were applied to determine whether the studies would be included in the analysis. In case of disagreement, decisions were made by consensus.

b. Study selection

Inclusion criteria were: French or English-language publication, adult patients with SSc, and at least 30 patients with SSc tested for LA, or/and aCL or/and anti-β2GpI. If the selected studies included overlapping cohorts during the same period, we chose to include 1 study per center (whichever study included the highest number of patient) based on the number of variables provided and aPL tested.

c. Data extraction

Following information were extracted from each selected studies: continent, country, center, ethnic origin of the patient, disease duration, sex ratio, cutaneous form (percentage of diffuse form), age of patients, percentage of patient with ILD and/or PAH and/or renal crisis, number of thrombosis or obstetric event, manufacturer of the ELISA assay, number of patient tested for aPL, number of patient positive for aPL

and which type of aPL (LA, aCL or anti-β2GpI).

III. Statistical analysis

Characteristics of patients were described using mean and standard deviation (SD) for continuous variables and count and percentage for categorical variables. Characteristics of patients as a function of aPL status (aPL+/aPL-) were compared using Student's test for continuous variables and Fisher's exact test for categorical variables. The associations between aPL status and vascular complications (arterial or venous thrombosis, miscarriage, pulmonary arterial hypertension, and digital ulceration) were studied using binomial logistic regressions. Adjustments were done (i) *a priori* for gender, age, SSc type (diffuse/limited) and tobacco history (yes/no), and (ii) for the characteristics that differed significantly between aPL+ and aPL- patients. Regression diagnostics were performed.

Similar analyzes were performed considering the titers of aCL and anti-β2GpI rather than the aPL status. Because these variables had a majority of zero values, they were categorized, as follows: (i) 0, ≥1 and <5, ≥5 and ≤20 UGPL/mL for aCL, and (ii) 0, ≥1 and <5, ≥5 and <10, ≥10 and ≤100 UA/mL for anti-β2GpI.

All statistical analyses were performed using R software, version 3.1.2 (R: A Language and Environment for Statistical Computing, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria); the threshold for statistical significance was set to $p < 0.05$.

RESULTS

I. Data from the cohort study

a. Patient and disease characteristics

The 249 patients were predominantly female (82%), with lcSSc (82%). Mean age of patients at the time of the study was 59.5 ± 13.3 years and mean disease duration was 10.7 ± 8.9 years (**Table 1**). The prevalence of ILD was 45%, digital ulcers 33%, PAH 6%. Forty-five (18%) patients had a history of arterial (n = 22) or venous (n = 29) thrombosis, and 40 (21%) patients had a history of miscarriage (**Tables S1 and S2**).

Table 1. Characteristics of the patients included in the study, and comparison between aPL positive or negative patients.

	All patients (n=249)	aPL – (n=233)	aPL + (n=16)	p
Sex, no. (%) female	205 (82)	191 (82)	14 (87)	1
Age, mean ± SD years	59.5 ± 13.3	59.1 ± 13.5	65.9 ± 7.3	**0.047**
Age at onset of disease, mean ± SD years	47.6 ± 13.7	47.6 ± 13.9	48.2 ± 11.5	0.897
Disease duration, mean ± SD years	10.7 ± 8.9	10.4 ± 8.8	15.3 ± 10.9	0.082
Disease subtype no. (%)				
Limited	203 (82)	188 (81)	15 (94)	0.318
Diffuse	46 (18)	45 (19)	1 (6)	
Pulmonary arterial hypertension, no. (%)	15 (6)	14 (6)	1 (6)	1
Interstitial lung disease, no. (%)	104 (45)	98 (46)	6 (38)	0.608
Digital ulceration, no. (%)	79 (33)	77 (35)	2 (14)	0.150
Renal crisis, no. (%)	1 (0)	1 (1)	0	1
BMI mean ± SD	25.1 ± 5.7	24.8 ± 5.4	29.2 ± 8.3	**0.003**
Tobacco use, no. (%)	99 (40)	97 (42)	2 (12)	**0.032**

ANA specificity, no. (%)				
ACA	139 (58)	127 (57)	12 (75)	0.196
Anti–topo I	50 (21)	47 (21)	3 (19)	1
Anti-RNA pol III	7 (3)	7 (3)	0	1
Anti–RNP	9 (4)	9 (4)	0	1
CRP no. (%)	21 (8)	20 (9)	1 (7)	1
Hypergammaglobulinemia no. (%)	30 (12)	29 (12)	1 (6)	0.701
HbA1c no. (%)	6 (2)	6 (3)	0	1
Arterial or venous thrombosis, no. (%)	45 (18)	39 (17)	6 (37)	0.086
Arterial thrombosis, no. (%)	22 (9)	19 (8)	3 (19)	0.160
Stroke/transient ischemic attack	11 (4)	9 (4)	2 (12)	0.154
Acute limb ischemia	3 (1)	3 (1)	0	1
Myocardial infarction	5 (2)	4 (2)	1 (6)	0.287
Venous thrombosis, no. (%)	29 (12)	24 (10)	5 (31)	**0.027**
DVT	22 (9)	18 (8)	4 (25)	**0.041**
PE	9 (4)	7 (3)	2 (12)	0.108
Miscarriage, no. (%)	40 (21)	35 (20)	5 (42)	0.136

ANA = antinuclear antibody; anti–topo I = anti–topoisomerase I antibody;

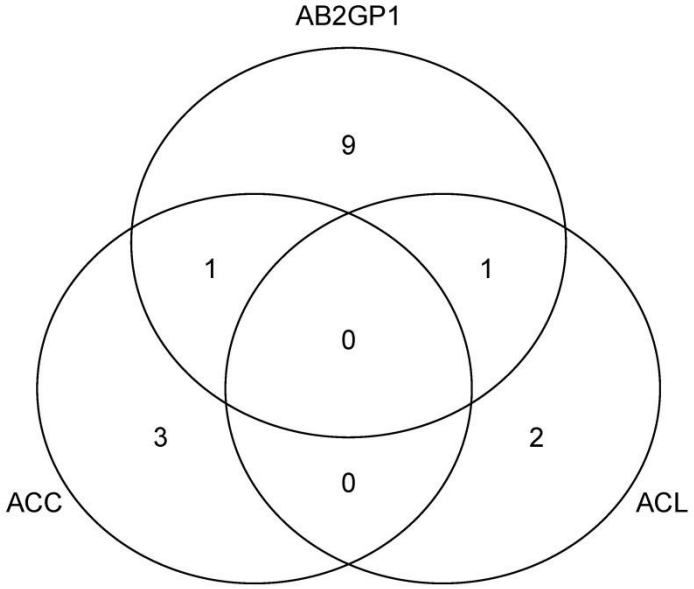
ACA = anticentromere antibody; Anti-RNA pol III = anti – RNA polymerase III antibody; Anti–RNP = Anti–RNP antibody. Age at onset of disease and disease duration are as of the first appearance of a non–Raynaud's phenomenon symptom. Tobacco use = ancient or active. CRP = C-reactive protein, patients with a CRP > 10 mg/L; HbA1c = patients with HbA1c > 6.5%. DVT = Deep venous thrombosis; PE = pulmonary embolism.

b. Frequency of aPL

One or more aPL were found in 16 (6.4%, 95%CI [3.4-9.5]) patients. One patient was positive for both LA and anti-β2GpI, and one was positive for aCL and anti-β2GpI. The prevalence of positive LA was 1.6%. The prevalence of aCL was 1.2% with a mean value of 14 UGPL/mL for positive patients. The prevalence of anti-β2GpI was 4.4% with a mean value of 28 UA/mL for positive patients (**Figure 1**).

Among patients with aPL, LA was found in 25%, aCL in 18.8%, and anti-β2GpI in 68.8% of patients with aPL positivity. Double positivity was seen in 12.5%. At the time of the study, 2 patients had been previously diagnosed with an APS based on clinical and biological criteria.

In our cohort, among the twenty-nine patients with a history of venous thrombosis, five had aPL positivity (LA and/or anti-β2GpI, but no aCL), two was diagnosed as having APS (they are the two positive for LA), and one had a pulmonary neoplasm. All these aPL positive patients have already been diagnosed SSc before thrombotic event. Three of them had experienced more than one thrombotic event, but we do not have enough criteria to classify them as APS.



	Prevalence in SSc cohort (%)	Frequency in SSc patients with aPL (%)
LA	1.6	25.0
aCL	1.2	18.8
Anti-β2GpI	4.4	68.8

Figure 1: Venn diagram representing distribution of aPL positivity among the SSc cohort. Prevalence of aPL in SSc cohort and frequency in patients with aPL

c. Associations with clinical manifestations

The associations of aPL with disease manifestations are presented in **Tables 1 and 2**. The sixteen patients with aPL were predominantly female (87%), with lcSSc (94%). Mean age of aPL positive patients was higher than aPL negative patient (65.9 years versus 59.1, p=0.047). A higher BMI (29.2 versus 24.8, p= 0.003) was found in aPL positive patients group. Smoking was less frequent in aPL positive patients group (12% versus 42%, p=0.032). No difference was found regarding disease subtype, ILD, DU, autoantibodies status, CRP elevation, HbA1c > 6.5%, hypergammaglobulinemia.

In univariate analysis, aPL positivity was associated with an increased risk of venous thrombosis (OR = 3.91; 95%CI [0.98-13.53]; p=0.027). No association was found between aPL positivity and arterial thrombosis, miscarriage, PAH, ILD, DU, and renal crisis.

When adjusted on sex, age, disease subtype, tobacco use, and BMI there was a trend toward an association between venous thrombosis and aPL positivity (OR = 3.24, 95%CI [0.42-8.52]; p = 0.064). aPL positivity was significantly associated with miscarriage (OR = 4.31; 95%CI [1.09-16.33]; p=0.031). No association was found between aPL positivity and arterial thrombosis, PAH, ILD, DU, and renal crisis (**Table 2**).

Table 2. Univariate and multivariate comparison of association between aPL positivity and clinical manifestations.

	univariate			multivariate		
	OR	95%CI	p	OR	95%CI	p
Arterial or venous thrombosis	2.92	0.82-9.50	0.085	2.59	0.77-8.12	0.108
Arterial thrombosis	2.56	0.43-10.54	0.160	2.14	0.87-10.9	0.307
Venous thrombosis	**3.91**	**0.98-13.53**	**0.027**	3.24	0.42-8.52	0.064
Miscarriage	2.84	0.67-11.11	0.136	**4.31**	**1.09-16.33**	**0.031**
Digital ulceration	0.31	0.03-1.47	0.150	0.45	0.07-1.86	0.327
PAH	0.97	0.02-7.26	1	0.73	0.04-4.43	0.776

d. Titers of aPL

Titers of aCL were all comprised between 10 and 40 UGPL/mL. Seven patients (63.6%) had titers of anti-β2GpI between 10 and 40 UA/mL, and four had titers over 40 UA/mL. (**Figure 2**, **Table 3**)

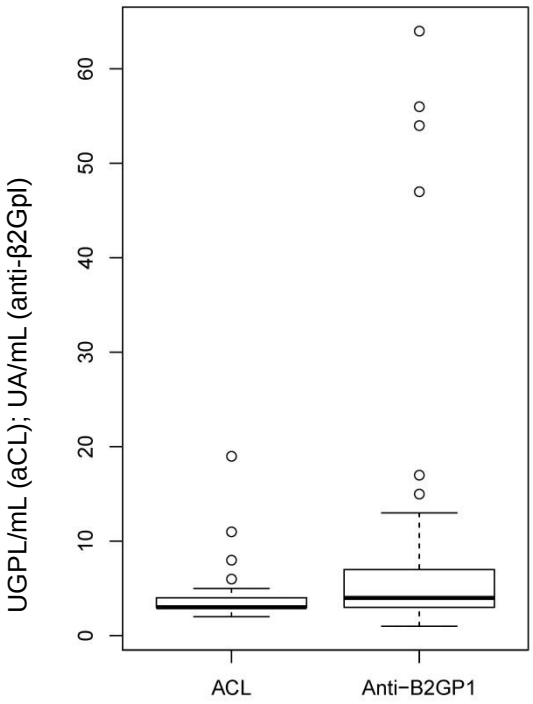


Figure 2: Titers of aCL and anti-β2GpI

Table 3. Types and titers of antiphospholipid antibodies.

Antibody titre U/ mL	Antibody type n. (%)	
	ACL IgG	anti-β2GpI
< 10	246 (98.8)	238 (95.6)
≥10	3 (1.2)	11 (4.4)

e. Clinical associations and antibodies titers

In multivariate analysis (model adjusted on sex, age, disease subtype, tobacco use, disease duration, gammaglobulin level, and anti-U1RNP positivity), there was an association between aCL titers (≥ 5 UGPL/mL) and venous thrombosis (OR 3.69; 95%CI [0.98-12.9]; p=0.043) or PAH (OR 6.35; 95%CI [1-41.1]; p=0.043).

Anti-β2Gp1 titer ≥ 10 UA/mL was associated with an increased risk of miscarriage (OR 5.25; 95%CI [1.04-27.1]; p=0.041) in multivariate analysis (model adjusted on sex, age, disease subtype, tobacco use, BMI, gammaglobulin level) (**Table 4, S3 and S4**).

Table 4. Multivariate comparison of association between aCL and anti-β2GpI titers and clinical manifestations.

	aCL (UGPL/mL)[1]					anti-β2GpI (UA/mL)[2]					
	[0,1[	[1,5[		[5,20]		[0,1[	[1,5[		[5,10[		[10,100]	
	Ref	Adjusted OR (CI)	p	Adjusted OR (CI)	p	Ref	Adjusted OR (CI)	p	Adjusted OR (CI)	p	Adjusted OR (CI)	p
Arterial or venous thrombosis		1.12 (0.50-2.44)	0.780	2.66 (0.83-8.10)	0.088		0.64 (0.25-1.52)	0.325	0.90 (0.27-2.66)	0.861	3.30 (0.80-13.3	0.090
Arterial thrombosis		0.84 (0.28-2.34)	0.739	1.52 (0.30-6.17)	0.576		0.47 (0.12-1.53)	0.235	0.59 (0.84-2.55)	0.526	3.29 (0.57-1.63)	0.153
Venous thrombosis		1.71 (0.67-4.36)	0.257	**3.69 (0.98-12.9)**	**0.043**		1.02 (0.35-2.83)	0.973	0.95 (0.20-3.43)	0.946	3.84 (0.81-16.7)	0.074
Miscarriage		1.43 (0.64-3.19)	0.381	0.62 (0.09-2.62)	0.557		0.94 (0.39-2.19)	0.888	0.56 (0.12-1.91)	0.395	**5.25 (1.04-27.1)**	**0.041**
Digital ulceration		0.66 (0.33-1.28)	0.226	1.85 (0.64-5.39)	0.253		0.60 (0.29-1.19)	0.150	0.83 (0.35-1.92)*		0.668*	
PAH		3.64 (0.94-17.9)	0.074	**6.35 (1-41.1)**	**0.043**		1.35 (0.41-4.42)	0.614	0.27 (0.01-1.77)*		0.250*	

(1) Adjusted on sex, age, disease subtype, tobacco use, disease duration, gammaglobulin level, anti-U1RNP positivity; (2) Adjusted on sex, age, disease subtype, tobacco use, BMI, gammaglobulin level; Ref: class reference; * class : [5,100]

II. Systematic review:

a. Studies included

1291 references were retrieved as result of search (575 articles in Pubmed and 716 in Embase). 79 articles were included for full text review after reading the titles and abstracts. Of these articles, 32 were assessed for eligibility. 8 articles were further excluded (essentially because of overlapping cohorts). Finally 24 studies were included in the review (**Figure 3**).

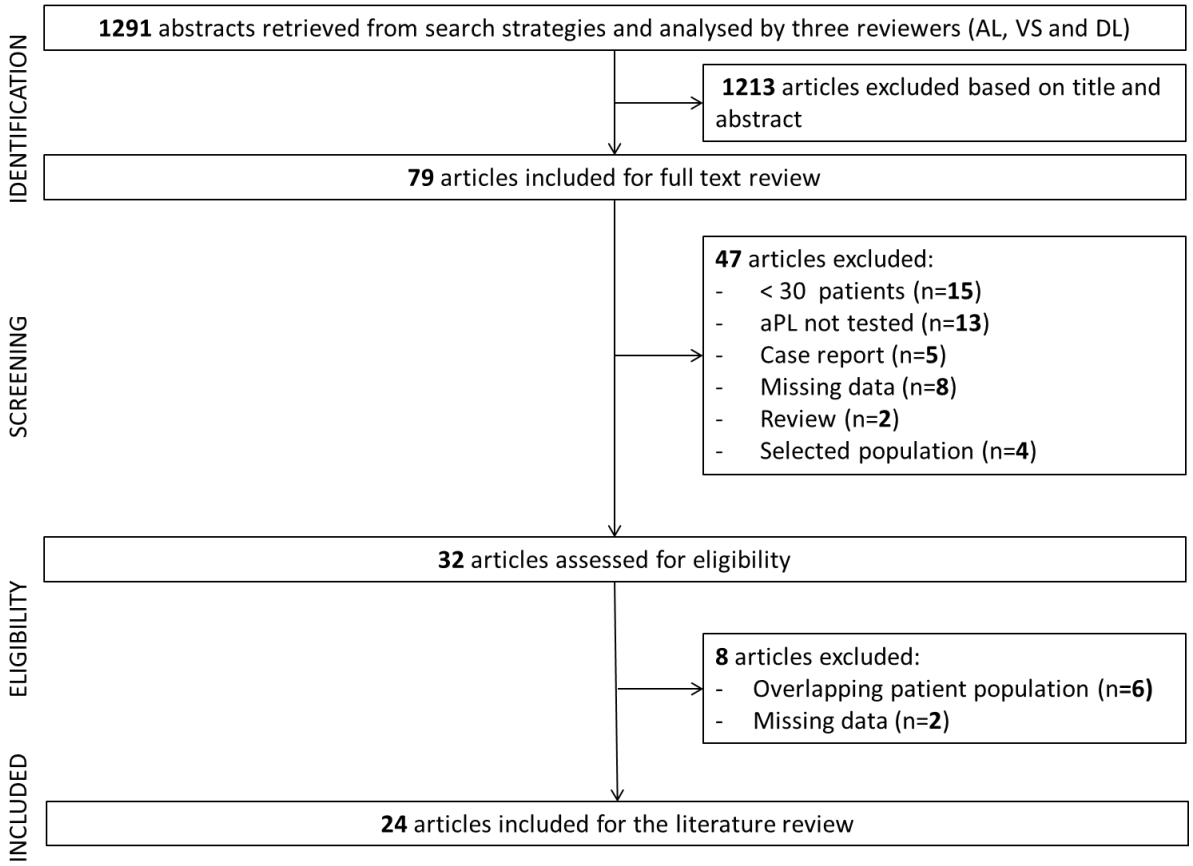


Figure 3. Flow chart showing the search strategy.

b. Prevalence of aPL

Prevalence of aPL positivity (one or more) in SSc in literature ranged from 0 to 58%. LA was found in 0 to 16% of patients; aCL in 0 to 34% and anti-β2GpI in 0 to 50% (**Table 5**).

c. Clinical associations

Six studies out of 11 found an association between aPL and PAH (aCL in 5, one or more aPL in 1). Two studies out of 8 found an association between aPL (aCL) and DU and 2 studies found a trend. One study out of 5 found an association between aPL (aCL and anti- β2GpI) and renal involvement; between aPL (aCL) and Raynaud phenomenon; between aPL (aCL) and ILD. One study out of 4 found an association between aPL (aCL) and thrombose. No study out of 3 found an association between aPL and miscarriage. No study out of 6 found an association between aPL and SSc subtype (**Table 5**).

Table 5. Prevalence and clinical associations in the literature

Authors, year	Countries	Number of patients	Prevalence (%)				Association
			LA	aCL	B2GpI	APL+	
Antonioli and al., 2003 (21)	Italy	60	NA	13	23	43	aCL and PAH Thrombosis
Assous and al., 2005 (22)	France	108	0	14	05	NA	aCL and PAH ; no association with disease subtype, Raynaud phenomenon, DU or ILD
Buchanan and al., 1989 (23)	Australia	35	NA	8.6	NA	8.6	NA
Enzenauer and al., 2006 (16)	USA	82	NA	14.6	NA	14.6	No association
Gupta and al., 2009 (15)	India	72	2.7	7	14	9.7	No association
Herrick and al., 1994 (24)	UK	68	NA	34	NA	34	NA
Ihn and al., 1996(12)	Japan	80	NA	25	NA	25	IgG β2GpI/aCL and PAH
Liberati and al., 2010 (25)	Brazil	54	16.6	NA	NA	16.6	No association with peripheral vascular manifestation

Lima and al., 1991 (26)	Spain	35	NA	0	NA	0	NA
Lise and al, 2010 (27)	France	55	NA	17	NA	17	NA
Manoussakis and al., 1987 (28)	Greece	40	NA	5	NA	5	NA
Marie and al., 2008 (11)	France	69	4.5	18.8	4.5	18.8	Association with PAH No association with DVT or miscarriage
Mellal and al., 2014 (29)	Algeria	147	NA	10.2	NA	10.2	aCL with PAH
Merkel and al., 1996 (30)	USA	45	NA	6.7	NA	6.7	NA
Mok and al., 2011 (31)	China	53	NA	NA	NA	13.2	NA
Morrisroe and al., 2014 (13)	Australia	940	0	NA	7	24	aCL (IgG) with PAH, digital ulcer; aCL (IgG and IgM) with ILD; aCL (IgM) with Raynaud phenomenon. No association with DVT or PE

Parodi and al., 2001 (32)	Italy	90	NA	12.2	3.3	12.2	Cutaneous ulcer and aCL
Picillo and al., 1997 (33)	Italy	105	NA	26.6	NA	26.6	No significant association
Pope and al., 2000 (34)	Canada	63	NA	4.8	NA	4.8	NA
Regéczy and al., 2000 (35)	Hungary	43	NA	NA	NA	2.3	NA
Renaudineau and al., 2001 (36)	Israel	478	NA	11.5	NA	11.5	NA
Tektonidou and al., 2000 (9)	Greece	30	NA	0	0	0	NA
Toure and al., 2013 (10)	Senegal	40	5	17.5	50	57.5	No association
Wielosz and al., 2009 (37)	Poland	50	NA	18	NA	56	aCL and anti- β2GpI with deterioration of renal function Other clinical manifestation untested

NA = not available

DISCUSSION

The main results of our study are as follows: 1) the prevalence of aPL in our cohort of SSc patients was 6.4% (respectively 1.6%, 1.2%, 4.4% for LA, aCL and anti-β2GpI), 2) there was an association between venous thrombosis and miscarriage and aPL positivity, 3) using the manufacturer cut-off levels, we did not find any association between aPL seropositivity and clinical manifestations of SSc such as DU or PAH, 4) higher levels of aCL corresponded to a higher likelihood of PAH, and higher levels of anti- β2GpI corresponded to a higher likelihood of miscarriage.

The prevalence of aPL in SSc in our study was 6.4% (95%CI [3.4-9.5]), which is rather low compared to previous reports. Although the prevalence of aPL ranges from 0 to 58% in the literature (9–13, 15, 16, 21, 23–26, 28–37), most of the studies reported an overall prevalence higher than 10%. For largest cohorts (>100 patients), the prevalence differed between 10.2 and 26.6%, which represents a smaller range (13, 29, 33, 36). Prevalence of aCL-IgG in literature varied from 0 to 25.5% (33, 38); prevalence of anti-β2GpI-IgG from 0 to 37.5% (9,10), but only Touré and al. reported a such higher prevalence, other studies found anti-β2GpI-IgG in SSc patients' sera in a range between 1.7 and 4.6% (11, 21, 22). We found a prevalence of 4.4% for anti-β2GpI, which is consistent with published data, and 1.2% for aCL, which is lower. Four patients were positive for LA in our study (1,2%), while two large cohorts had none (13, 22). Variations of aPL positivity between studies may results from methodological differences: different positivity cutoff, different type of aPL tested and subgroups analysis, size of cohort. This heterogeneity could also be explained by the

geographic origin of the study, because of potential genetic or environmental factors. Indeed, Touré and al. reported the highest prevalence in the review of literature, with an African ethnicity cohort (10); while studies from North and South America found a prevalence range of 4.8 to 16.6% (16, 25, 30, 34).

LA was found in 25% of patient with aPL positivity, aCL in 18.8%, and anti-β2GpI in 68.8% in our cohort. Double positivity was seen in 12.5%. Prevalence of each of these antibodies in PAPS is heterogeneous in literature (**Table 6**).

Cervera and al. described a population of PAPS and SAPS, but they did not find any difference in aPL frequencies between the two populations.

Compared to PAPS, in our aPL positive patients, the prevalence of LA is in the lower range, prevalence of aCL and double positivity are lower than all existing literature; and prevalence of anti-β2GpI is in the higher range.

Table 6. Prevalence of LA, aCL(IgG) and anti-β2GpI (IgG) in literature.

	Number of patient	LA (%)	Anti-β2GpI (%)	aCL (%)	> 1 aPL
Danowski A (39)	17	100	NA	77	NA
Mondejar R (40)	26	58	38	50	NA
Ahluwalia J (41)	47	23	68	49	Simple 40 Double 43 Triple 17
Turiel M (42)	56	86	NA	64	
Veres K (43)	105	29	76 (IgG & IgM)	50	NA
Gómez-Puerta JA (44)	128	65	NA	86	NA
Stojanovich L (45)	162	54	32	33	60
Djokovic A (46)	260	51	32	36	60
Cervera R (47)	1000	54	NA	76	Double 42

NA: not available

We found an association between aPL positivity and venous thrombosis in univariate and a trend in multivariate analysis. This association is not common in studies of SSc patients, most of them did not find an increased risk of venous thrombosis in case of aPL positivity (10, 11, 13, 15, 16). Antonioli and al. reported an association between thrombosis (arterial or venous) and aPL positivity (21). In APS, it has been reported that the IgG isotype was the only one associated with thrombosis (48). Of note, most of the previous studies have analyzed the association between merged IgG and IgM isotype and deep venous thrombosis or pulmonary embolism.

This study showed an increased risk of miscarriage in case of aPL positivity, and this association was significant in multivariate analysis. To our knowledge, this association has never been reported in literature. Only a few studies have analyzed the correlation between aPL positivity in SSc and fetal loss or spontaneous abortion, in smaller cohorts and did not find any association (11, 16, 49). One of our patients with miscarriage history was known to have an APS. The 3 other patients with miscarriage history and aPL positivity could not be classified retrospectively as having an APS (we did not have precise history of miscarriage and only one positive sample). Mean age at onset of the disease was 47.6 years, meaning that most patients had already finished childbearing at that time. In our cohort, SSc had been diagnosed after the time of miscarriage (more than 2 years after) in 25/37 women who had experienced miscarriage. In the few studies that have evaluated an association between aPL positivity and miscarriage (11,16,49), the tested antibodies were heterogeneous (aCL IgG and IgM isotype / only aCL IgG isotype / aCL and anti-β2GpI IgG and IgM isotype and LA). However it is probable that IgG isotype for both aCL and anti-β2GpI are more robustly associated with clinical events. The lack of association in the previous studies may be linked with a lack of power due to the smaller sample size of these studies.

Using the manufacturer cut-off levels, we did not show any association between aPL positivity and clinical manifestations, in particular with PAH and DU. This may be due to a lack of power (small number of events). Regarding the association between aPL positivity and PAH, results in literature are debated. Marie and al. reported an increased risk of PAH in case of one or more aPL positivity (11). Antonioli and al., Assous and al. and Morrisroe and al. reported an association between aCL positivity and PAH (13, 21, 22), while Boin and al. found this association with anti-β2GpI (14)

in a selected population. On the other hand Gupta and al., Enzenauer and al., did not report this association (10, 15, 16). Touré and al. found a trend (10). As in our cohort, studies that did not highlight an association between PAH and aPL positivity had a low prevalence of aPL (9.1 to 14%) (15, 16).

Interestingly, considering the titers of aPL rather than the aPL status positivity/negativity, we found that higher titers of aCL ≥5 UGPL/mL were associated with PAH. This is consistent with the existing literature. Morrisroe and al. identified that higher titers of aCL-IgG corresponded with a higher risk of PAH (13) in SSc patients. Assous and al. identified a trend towards an association between a higher mean titer of aCL and PAH (p=0.06) (22). Stojanovich and al. reported the same association in patients with SAPS (50).

We reported a significant association between higher titers of anti-β2GpI and risk of miscarriage. To our knowledge, there is no previously published data on these findings in SSc. This result might be linked with the pathogenic role of aPL in fetal loss observed in APS. Indeed, aPL (in particular β2GpI-dependant antibodies) bind to human trophoblasts and affect several cell function in vitro (51). However, in the PROMISSE (Predictors of pRegnancy Outcome: bioMarkers In antiphospholipid antibody Syndrome and Systemic lupus Erythematosus) study of lupus patients, no association was found between adverse pregnancy outcomes and anti-β2GpI (52).

Comparing our data with those in PAPS, we found in aPL positive SSc patients a venous thrombosis prevalence of 31.3%, which is in range of previous published data (25.9 to 50%) (41, 45, 53). DVT and PE occurred in 25% and 12.5% respectively which is in the low range of literature (31.7 to 48%, and 9 to 29%) (47, 54). Regarding arterial thrombosis events, they occurred in 18.8% of our patients, which

is lower than in PAPS (34.6 to 40.4%) (41, 45). We found a prevalence of 6.3% of myocardial infarction, which is similar to literature (2.8 to 8%) (45,47,54). 12.5% of our patients had a stroke or a transient ischemic attacks history. In the literature, stroke are observed in 7 to 23% of patients and transient ischemic attacks in 13.1 to 26% (47,54). Similarly to the literature on PAPS and thrombosis events, DVT was the most frequent event in our cohort, followed by PE, stroke or transient ischemic attack, then by myocardial infarction. Miscarriage occurred in 41.7% of our patients. Previous reports are heterogeneous. It seems to be higher than in PAPS (between 10 and 23.2%, in most of the studies, 55% in one study) (42,45,47,53). However it does not seem that SSc is associated with an increased risk of miscarriage. Moreover, the mean age at diagnosis was around the age of 50 years, so women had ending time of child bearing.

In general population aPL prevalence ranged between 0 to 5% (55), and until 50% (low titers of aCL) in elderly population (55). We found that mean age of our aPL positive patients is higher than aPL negative patients. It has been reported that high titers of aCL are associated with incidence of thrombosis in PAPS (39). Titers of aCL were rather low in our study. Miyakis and al. set as threshold for positive aPL >40 GPL or 99th percentile. Our threshold for positive aCL and anti-β2GpI were ≥10 UGPL/mL and ≥10UA/mL respectively. Definition of the level that best correspond to the risk of clinical manifestation is difficult to establish (6).

Precise physiopathology of both APS and SSc remains unclear, but in both of these diseases, endothelial cells seem to be involved. Assous and al. reported a significant correlation between aCL (IgG) titer and the amount of von Willebrand factor produced in SSc. They also found an association between patients with PAH and the amount of

von Willebrand factor produced (22). It has been show that endothelial cell injury in SSc patients was accompanied by an elevation in the level of von Willebrand factor (56). This suggests that aCL positivity could be associated with endothelial injury and PAH. It has also been reported that there were an increased amount of E-selectin in patients with aPL positivity (with or without APS), and an increased amount of P-selectin in patient with APS (57). Other authors reported increased amount of sVCAM-1 in PAPS (58). These three molecules are also involved in pathogenesis of SSc (1, 59). These pathways might be potentially involved in the mechanisms of microthrombosis in SSc, and thrombosis in APS.

aCL (but only IgM isotype) have been associated with atherosclerosis, which is another data for the role of APLA in vascular diseases (60). It has also been reported that anti-β2GpI are independently associated with myocardial infarction in premenopausal women, without atherosclerosis (61). We found that patients with aPL positivity and a VT history developed VT after the onset of the disease, suggesting that aPL might play a role in thrombosis pathogenesis among these patients. Some data suggested a common pattern of reactivity with both micro and macro endothelial cell in APS in accordance with the vascular clinical manifestations of the disease (62). Lauwerys and al. reported two cases of SSc patients myocardial microangiopathy associated with aPL (63). As in APS, SSc clinical manifestations attest of micro and macrovascular involvement.

However, as in SSc, it remains unknown whether aPL positivity constitutes a primary event or is merely a secondary event resulting from endothelial abnormalities. SSc might be the trigger (“second hit”) of thrombosis in presence of aPL. It has been reported that in APS, IgG isotype were the only associated with thrombosis and obstetrics events (48). It remains unknown whether IgM and IgA isotypes may have a pathogenic role in other conditions than APS.

Pulmonary hypertension is not a common clinical manifestation in APS (2.2% in Cervera and al., 3.5% in PAPS in Vianna and al. (47, 53)), and most of the time is secondary to pulmonary embolism. PAH has been described frequently in APS associated with lupus, therefore it is difficult to establish if it was related to aPL, lupus, or both of them (64). Regarding obstetrics complications, two cases of fetal loss in SSc patients with aCL antibodies reported in literature described small fibrotic placentas with a high frequency of decidual vascular abnormalities and infarction (65). However in these two cases, patients were known as having a diffuse SSc, and fulfilled criteria of APS. As we said before, most of our patients who experienced miscarriage in case of aPL positivity, were diagnosed SSc years later.

Our study has several limitations. First, we chose to quantify only IgG subtype of aCL and anti-β2GpI, because IgG was the most prevalent isotype among patient with thrombosis and fetal loss in APS, and the only one associated with these events (48). However an overall screening of anti-β2GpI IgG, IgM and IgA subtype was done. IgM and IgA were often more prevalent in the studies in which they are quantified (13, 14). Secondly, due to the low prevalence of aPL, there might be a lack of power. Thirdly thrombosis history and miscarriage has been collected retrospectively, leading to a potential memorization bias and a selection bias (in classification of APS patients). It is difficult to avoid this bias, because obstetrical events occurred mainly years before the onset of SSc. A prospective study would be needed to avoid these biases.

In conclusion, this study found a low prevalence of aPL in SSc (6.4% 95%CI [3.4-9.5]). We reported an association between VT and aPL positivity. This association

was not confirmed in multivariate analysis. We found an increased risk of miscarriage in case of aPL positivity. We did not highlight any association between aPL positivity and clinical manifestations of SSc (PAH, digital ulceration…). However we identified an association between high titers of aCL and PAH, and between high titers of anti-β2GpI and miscarriage. These data provide additional insights into the vascular involvement of SSc. The systematic review showed that the prevalence of aPL in SSc was highly variable (range 0-58%). Clinical manifestations associated with aPL positivity were more frequently PAH. A meta-analysis will complete this systematic review and might confirm the findings of our cohort or highlight new unknown associations.

CONCLUSION GENERALE

Notre travail retrouve une prévalence des anticorps anti-phospholipides dans la sclérodermie systémique de 6.4% (IC95 [3.4-9.5]), qui est inférieure à ce qui est rapporté dans la littérature. Toutefois, si on compare à la prévalence des isotypes IgG, notre résultat est semblable à ceux de la littérature. En effet la revue de la littérature témoigne de l'hétérogénéité des travaux effectués sur le sujet, et il est difficile d'en tirer des conclusions.

Nous avons mis en évidence une association entre la présence d'aPL et la survenue de thrombose veineuse, ce qui est rarement rapporté dans la littérature. Toutefois ce résultat n'est pas confirmé en analyse multivariée, et mériterait des analyses complémentaires. Nous avons également mis en évidence une association entre la présence d'aPL et la survenue de perte fœtale, bien que les premiers signes de la maladie surviennent après ces événements la plupart du temps. Cette association n'a jamais été rapportée dans la littérature, mais est rarement recherchée.

En revanche nous n'avons pas mis en évidence d'association entre la présence d'aPL et les manifestations cliniques de la ScS, notamment avec l'HTAP. Cependant nous retrouvons une association entre le taux d'aCL (taux les plus élevés) et la présence d'HTAP. Nous pouvons donc émettre l'hypothèse que ces résultats sont liés à un manque de puissance, même si cette association n'est pas unanimement rapportée dans la littérature. Il serait intéressant de mener une étude sur une cohorte importante, d'analyser les associations cliniques avec chaque type et isotype (IgG ou IgM) d'anticorps, ainsi qu'avec la simple, double ou triple positivité.

Sur le plan physiopathologique le rôle des cellules endothéliales semble important, et

la présence des APL est probablement un témoin de l'atteinte vasculaire. Il reste à déterminer s'il s'agit d'un facteur pathogène ou uniquement d'un biomarqueur. Il pourrait en effet être intéressant de déterminer si la présence d'aPL est le témoin d'un phénotype particulier, à risque vasculaire plus élevé (tant sur le plan de l'HTAP que du risque thrombotique veineux), ces données ayant une implication clinique directe. Une méta-analyse viendra prochainement compléter la revue systématique de la littérature et pourrait confirmer les résultats de notre cohorte voire mettre en évidence de nouvelles associations cliniques encore inconnues.

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ANNEXES

Annexe 1 :

Table S1. Characteristics of thrombotic events

Patient and sex		SSc subtype	Thrombotic event	Disease duration at the time of the event (years)	LA	aCL (UGPL/ mL)	Anti - β2GP1 (UA/mL)	Time between event and aPL testing (years)	Age at the event (years)	Comments
1	F	lSSc	PE	+ 9	neg	0	0	3	71	
2	**F**	**lSSc**	**PE**	**+ 7**	**pos**	**5**	**56**	**9**	**50**	**APS**
3	F	lSSc	DVT1 DVT2	- 37 - 33	neg	3	4	40 36	22 26	
4	F	lSSc	DVT	0	neg	0	0	27	53	
5	F	lSSc	DVT MI	- 5 + 8	neg	4	4	27 3	62 75	
6	F	lSSc	PE BTUA	+1 0	neg	0	0	2 3	41 40	cryoF estrogen contraception
7	F	lSSc	DVT	nk	neg	0	0	nk	nk	Pregnancy, surgery, smoker
8	F	lSSc	3 DVT	nk	neg	4	3	nk	nk	Smoker
9	M	lSSc	3 DVT	nk	neg	3	0	nk	nk	Protein C deficiency smoker

10	F	lSSc	DVT	nk	neg	0	0	nk	nk	
11	**F**	**lSSc**	**DVT1** **DVT2** **DVT 3** **Stroke/TIA**	**+ 5** **+ 6** **+ 7** **- 3**	**neg**	**5**	**47**	**2** **1** **0** **10**	**65** **66** **67** **57**	**Pulmonary neoplasm**
12	**F**	**lSSc**	**DVT**	**0**	**pos**	**0**	**4**	**17**	**54**	**APS**
13	F	lSSc	DVT	- 12	neg	3	3	41	28	Pregnancy, hypergamma
14	**F**	lSSc	**DVT1** **4 DVT, 1 PE** **MI**	**+ 18** **nk** **+ 19**	**neg**	**4**	**15**	**3** **nk** **2**	**65** **nk** **66**	
15	F	lSSc	DVT	+ 20	neg	4	6	11	35	thoracic outlet syndrome
16	F	lSSc	DVT 1 DVT 2	+ 14 + 28	neg	5	4	15 1	58 72	Surgery, hypergamma
17	F	dSSc	DVT	+ 15	neg	0	0	5	67	
18	F	lSSc	DVT	+ 3	neg	0	0	4	67	
19	**F**	lSSc	**DVT 1** **DVT 2**	**- 24** **+ 3**	**neg**	**4**	**12**	**30** **3**	**26** **53**	**Postpartum, smoker**
20	F	lSSc	DVT Stroke/TIA	+ 1 nk	neg	0	0	0 nk	65 nk	smoker cardiopathy
21	F	lSSc	DVT	+ 1	neg	3	5	3	31	Immobilization (trauma)
22	F	lSSc	PE	nk	neg	0	0	nk	nk	

23	H	lSSc	DVT ALI	nk 0	neg	3	3	nk 18	nk 39	cryo, Hammer syndrome, smoker
24	F	lSSc	DVT	- 40	neg	0	0	40	37	smoker
25	F	lSSc	PE	+ 17	na	0	9	3	61	
26	F	lSSc	PE	+ 1	neg	8	6	18	61	
27	F	lSSc	DVT1, PE 1 DVT2, PE 2	- 10 + 13	neg	5	0	25 2	34 57	Postpartum, surgery, thrombocytosis (splenectomy)
28	F	lSSc	DVT 1 DVT2 DVT 3	- 29 - 24 - 4	neg	0	7	34 29 9	35 40 60	One surgery
29	F	lSSc	PE	+ 6	neg	3	0	12	56	HRT
30	F	dSSc	PE	+ 16	neg	0	3	1	39	Portacath surgery smoker
31	F	lSSc	DVT	+ 5	neg	4	0	4	72	
32	F	lSSc	ALI	0	neg	0	0	4	52	cryoF, smoker
33	M	lSSc	Stroke/TIA	+ 8	neg	0	0	1	68	smoker
34	M	lSSc	BTUA	0	neg	0	5	1	59	Link to SSc smoker
35	M	lSSc	ALI	nk	neg	4	7	nk	nk	Smoker, obesity, dyslipidemia, arterial hypertension,

36	F	dSSc	MI, Stroke/TIA	+ 7	na	0	0	1	73	Atrial myxoma, breast cancer smoker
37	**F**	lSSc	**Stroke/TIA**	**- 3**	**neg**	**19**	**17**	**8**	**50**	
38	F	lSSc	Stroke/TIA 1 Stroke/TIA 2	+ 7 + 10	neg	3	0	10 7	34 37	Smoker hypergamma
39	F	lSSc	Stroke/TIA	- 17	neg	5	0	32	36	Neurinoma surgery
40	F	lSSc	Stroke/TIA	+ 15	neg	0	7	8	74	none
41	F	lSSc	BTUA	+ 16	neg	3	3	1	68	Link to SSc
42	F	lSSc	Stroke/TIA	+ 8	neg	0	0	1	72	
43	F	lSSc	MI	+ 29	neg	3	4	6	76	dyslipidemia, arterial hypertension,
44	F	lSSc	MI	- 19	neg	0	0	25	56	
45	F	lSSc	Stroke/TIA	+ 16	neg	3	0	1	74	
46	F	lSSc	Stroke/TIA	+ 29	neg	0	0	1	59	

lSSc = limited SSc ; dSSc = diffuse SSc ; PE = pulmonary embolism ; DVT = deep venous thrombosis ; MI = myocardial infarction; BTUA = Bilateral thrombosis of ulnar arteries; Stroke/TIA = stroke or transient ischemic attack; ALI = acute limb ischemia; pos = positive; neg = negative; ACL and anti-β2GP1 positivity ≥ 10; nk = not known; cryo F = cryofibrinogen, cryo = cryoglobulinemia; HRT = Hormone remplacement therapy. Disease duration at the time of the event (years) : + = diagnosis prior the event; - = diagnosis posterior at the event; 0 = simultaneous.

Annexe 2 : Table S2. Patients' characteristics according to thrombosis or obstretric history.

	Arterial thrombosis		Venous thrombosis		Miscarriage	
	no	yes	no	yes	no	yes
Sex, no. (%) female	185 (82)	18 (82)	178 (81)	27 (93)	na	na
Age, mean (SD) years	**59 (13)****	**67 (11)****	**59 (13)***	**64 (11)***	59 (15)	57 (11)
Age at onset of disease, mean (SD) years	47 (14)	52 (12)	47 (14)	50 (15)	46 (14)	46,5 (12)
Disease duration, mean (SD) years	10,4 (8,9)	12,7 (8,7)	10,4 (9)	13,3 (8)	11,6 (9,2)	9,8 (7,4)
Disease subtype						
Limited	180 (80)$^{\theta}$	21 (95)$^{\theta}$	176 (80)	27 (93)	129 (88)$^{\theta}$	30 (75)$^{\theta}$
Diffuse	45 (20)	1 (5)	43 (20)	2 (7)	18 (12)$^{\theta}$	10 (25)$^{\theta}$
Pulmonary arterial hypertension, no. (%)	15 (7)	0	11 (5)$^{\theta}$	4 (14)$^{\theta}$	8 (6)	2 (6)
Interstitial lung disease, no.	93 (45)	11 (50)	92 (46)	11 (41)	53 (40)	17 (46)
Digital ulceration, no. (%)	72 (34)	6 (30)	71 (34)	8 (31)	44 (31)	15 (38)
Renal crisis, no. (%)	0$^{\theta}$	1 (5)$^{\theta}$	1 (1)	0	1 (1)	0
BMI	25 (5,7)	26 (6,3)	24,9 (5,6)	26.5 (5,7)	25,3 (5,3)	25,2 (7,3)
Tobacco	88 (39)	9 (41)	90 (41)	8 (29)	52 (35)	15 (37)
ANA specificity, no. (%)						
ACA	124 (58)	13 (59)	**117 (56)***	**22 (79)***	92 (66)	25 (64)
Anti–topo I	45 (21)	5 (23)	44 (21)	5 (18)	29 (21)	4 (10)
Anti-RNA pol III	7 (3)	0	7 (3)	0	4 (3)	1 (3)
Anti–RNP	8 (4)	1 (5)	8 (4)	1 (4)	7 (5)	2 (5)
CRP (> 10 mg/L)	19 (8)	2 (9)	17 (8)	4 (14)	12 (8)	4 (10)
Hypergammaglobulinemia	29 (13)	1 (5)	28 (13)	2 (7)	13 (9)	7 (17)
HbA1c (> 6.5%)	5 (2)	1 (5)	3 (1)	2 (7)	3 (2)	1 (3)
Rheumatoid factor						
APL	13 (6)	3 (14)	**11 (5)***	**5 (17)***	7 (5)	5 (12)
IgG ACL	2 (1)	1 (5)	3 (1)	0	2 (1)	0
IgG anti-B2GpI	8 (4) $^{\theta}$	3 (14) $^{\theta}$	**7 (3)***	**4 (14)***	4 (3)$^{\theta}$	4 (10)$^{\theta}$
LA	4 (2)	0	2 (1)$^{\theta}$	2 (7)$^{\theta}$	2 (1)	2 (5)

$^{\theta}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	PAH		UD	
	no	yes	no	yes
Sex, no. (%) female	181 (83)	13 (87)	131 (83)	65 (82)
Age, mean (SD) years	59 (13)	65 (10)	61 (12)$^{\theta}$	57 (14)$^{\theta}$
Age at onset of disease, mean (SD) years	48 (14)	49 (12)	49 (13)$^{\theta}$	44 (15) $^{\theta}$
Disease duration, mean (SD) years	10,7 (9)	13,8 (9,1)	10,3 (9)	12,3 (8,9)
Disease subtype				
Limited	184 (84)	10 (67)	**157 (89)***	**55 (70)***
Diffuse	34 (16)	5 (33)	**18 (11)***	**24 (30)***
Pulmonary arterial hypertension, no. (%)	Na	na	8 (5)	7 (9)
Interstitial lung disease, no.	87 (43)	10 (67)	**53 (37)****	**40 (56)****
Digital ulceration, no. (%)	68 (33)	7 (47)	na	na
Renal crisis, no. (%)	1 (1)	0	1 (1)	0
BMI	25 (5,6)	24,9 (7,6)	**25,7 (5,8)***	**23,7 (5)***
Tobacco	**91 (42)***	**2 (13)***	62 (39)	33 (42)
ANA specificity, no. (%)				
ACA	124 (60)	7 (47)	**98 (65)***	**37 (49)***
Anti–topo I	41 (20)	5 (33)	**22 (15)***	**22 (29)***
Anti-RNA pol III	6 (3)	0	4 (3)	3 (4)
Anti–RNP	7 (3)	1 (7)	8 (5)	1 (1)
CRP (> 10 mg/L)	20 (9)	1 (7)	15 (10)	5 (6)
Hypergammaglobulinemia	25 (11)	3 (20)	15 (10)$^{\theta}$	14 (18)$^{\theta}$
HbA1c (> 6.5%)	6 (3)	0	5 (3,52)	1 (1,4)
Rheumatoid factor				
APL	15 (7)	1 (7)	12 (8)	2 (3)
IgG ACL	3 (1)	0	1 (1)	2 (3)
IgG anti-B2GpI	10 (5)	1 (7)	**9 (6)***	**0***
LA	4 (2)	0	4 (3)	0

$^{\theta}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Annexe 3 :

Table S3. Correlation between aCL titers and clinical manifestations

	aCL (UGPL/mL)			
	[0,1[n=137	[1,5[n=91	[5,20] n=21	p
Sex, no. (%) female	114 (83)	73 (80)	18 (86)	0.818
Age, mean ± SD years	58.6 (13.1)	60.6 (13)	60.5 (14.9)	0.504
Age at onset of disease, mean ± SD years	48.4 (13.4)	47.55 (13.9)	42.69 (14.8)	0.320
Disease duration, mean ± SD years	9.19 (7.8)	12.11 (9.6)	14.71 (11.2)	0.016
Disease subtype, no. (%)	113 (82)	73 (80)	17 (81)	0.880
Limited	24 (18)	18 (20)	4 (19)	0.880
Diffuse				
Pulmonary arterial hypertension, no. (%)	3 (2)	9 (11)	3 (16)	0.007
Interstitial lung disease, no. (%)	61 (49)	34 (40)	9 (43)	0.406
Digital ulceration, no. (%)	46 (35)	23 (26)	10 (53)	0.079
Renal crisis, no. (%)	1 (1)	0 (0)	0 (0)	1
BMI mean ± SD	24.5 (5.2)	25.7 (6.3)	26.6 (5.6)	0.132
Tobacco use, no. (%)	57 (42)	36 (40)	6 (29)	0.545
ANA specificity, no. (%)				
ACA	55 (42)	36 (41)	8 (38)	0.96
Anti–topo I	27 (21)	17 (20)	6 (29)	0.619
Anti-RNA pol III	5 (4)	2 (2)	0 (0)	0.847
Anti–RNP	1 (1)	7 (8)	1 (5)	0.014
CRP>10, no. (%)	11 (8)	7 (8)	3 (14)	0.567
Hypergammaglobulinemia, no. (%)	11 (8)	17 (19)	2 (10)	0.050
HbA1c>6.5, no. (%)	2 (1)	4 (4)	0(0)	0.341

Arterial or venous thrombosis, no. (%)	21 (16)	17 (19)	7 (33)	0.142
Arterial thrombosis, no. (%)	11 (8)	8 (9)	3 (14)	0.583
Stroke	6 (4)	2(2)	3(14)	0.064
Ischemia	1 (1)	2(2)	0 (0)	0.668
Myocardial infarction	2 (1)	3(3)	0 (0)	0.613
Venous thrombosis, no. (%)	12 (9)	12 (13)	5 (24)	0.095
DVT	9 (7)	10 (11)	3(14)	0.280
PE	3 (2)	3 (3)	3 (14)	0.044
Miscarriage, no. (%)	21 (20)	17 (26)	2 (13)	0.521

Annexe 4 :

Table S4. Correlation between anti-β2GpI titers and clinical manifestations

	anti-β2GpI (UA/mL)				
	[0,1[n=120	[1,5[n=83	[5,10[n=35	[10,100] n=11	p
Sex, no. (%) female	100 (83)	65 (78)	30 (86)	10 (91)	0.683
Age, mean ± SD years	59.6 (12.7)	59 (14.5)	58.8 (13.5)	65.4 (7.1)	0.495
Age at onset of disease, mean ± SD years	47.6 (12.9)	47.5 (14.7)	48.1 (15.3)	48 (8.7)	0.997
Disease duration, mean ± SD years	10.9 (9.5)	10.2 (8)	10.5 (9)	13.6 (9.9)	0.808
Disease subtype, no. (%)					
Limited	97 (81)	62 (75)	33 (94)	11 (100)	0.027
Diffuse	23 (19)	21 (25)	2 (6)	0(0)	0.027
Pulmonary arterial hypertension, no. (%)	7 (6)	7 (9)	0(0)	1 (9)	0.243
Interstitial lung disease, no. (%)	57 (52)	32 (42)	12 (36)	3 (27)	0.198
Digital ulceration, no. (%)	43 (38)	23 (29)	13 (38)	0 (0)	0.074
Renal crisis, no. (%)	1 (1)	0 (0)	0 (0)	0 (0)	1
BMI mean ± SD	24.8 (5.6)	25.1 (5.4)	25.0 (6.0)	29.6 (7.2)	0.059
Tobacco use, no. (%)	50 (42)	37 (45)	12 (34)	0 (0)	0.019

ANA specificity, no. (%)					
ACA	61 (54)	45 (56)	23 (68)	10 (91)	0.065
Anti–topo I	19 (17)	24 (30)	6 (18)	1 (9)	0.117
Anti-RNA pol III	5 (4)	2(3)	0 (0)	0 (0)	0.761
Anti–RNP	4 (4)	5 (6)	0 (0)	0 (0)	0.526
CRP>10, no. (%)	10 (8)	9 (11)	1 (3)	1 (9)	0.534
Hypergammaglobulinemia, no. (%)	13 (11)	12 (14)	5 (14)	0 (0)	0.593
HbA1c>6.5, no. (%)	2 (2)	4 (5)	0 (0)	0 (0)	0.43
Arterial or venous thrombosis, no. (%)	24 (20)	9 (11)	7(21)	5 (45)	0.038
Arterial thrombosis, no. (%)	12 (10)	4 (5)	3 (9)	3 (27)	0.099
Stroke	8 (7)	0 (0)	1 (3)	2 (18)	0.009
Ischemia	1 (1)	1 (1)	1 (3)	0 (0)	0.603
Myocardial infarction	2 (2)	2 (3)	0 (0)	1 (9)	0.348
Venous thrombosis, no. (%)	14 (12)	7 (9)	4 (11)	4 (36)	0.096
DVT	9 (8)	7 (8)	3 (9)	3 (27)	0.195
PE	6 (5)	0 (0)	1 (3)	2 (18)	0.016
Miscarriage, no. (%)	19 (21)	13 (22)	4 (15)	4 (50)	0.237

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Titre de la Thèse : Prévalence et associations cliniques des anticorps anti-phospholipides dans la sclérodermie systémique : données d'une cohorte lilloise et revue systématique de la littérature

Thèse - Médecine - Lille 2016

Cadre de classement : Médecine

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Mots-clés : sclérodermie systémique, anticorps anti-phospholipides, syndrome des anti-phospholipides, thromboses, perte fœtale, hypertension artérielle pulmonaire

Résumé :

Contexte. Des anticorps anti-phospholipides (aPL) peuvent être retrouvés dans le sérum des patients sclérodermiques. Des variations importantes de leur prévalence sont observées dans la littérature. Leurs associations cliniques restent largement méconnues. Cette étude avait pour objectifs de déterminer la prévalence des aPL dans une cohorte de patients sclérodermiques, d'évaluer leurs associations cliniques et de réaliser une revue systématique de la littérature.

Méthode. Dans notre centre, une recherche d'anticoagulant lupique, d'anticorps anti-cardiolipine (aCL) et d'anticorps anti-β2glycoproteine I (anti-β2GpI) a été réalisée chez 249 patients sclérodermiques de manière consécutive. Les associations cliniques ont été étudiées. Une revue systématique de la littérature a été réalisée via les bases Embase et PubMed.

Résultats. Au moins un anticorps était présent chez 16 patients (6.4% IC95 [3.4-9.5]). La présence d'aPL était associée aux thromboses veineuses (TV) en analyse univariée (OR = 3.91; IC95 [0.98-13.53]; p=0.027), et une tendance statistique était retrouvée en analyse multivariée (OR 3.24; IC95 [0.87-10.9]; p=0.064). En analyse multivariée, la présence d'aPL était associée aux pertes fœtales (OR 4.31; IC95 [1.09-16.33]; p=0.031), les titres élevés d'aCL (≥5 UGPL/mL) étaient associés à l'hypertension artérielle pulmonaire (HTAP) (OR 6.35; IC95 [1-41.1]; p=0.043) et aux thromboses veineuses (OR 3.69; IC95 [0.98-12.9]; p=0.043). Les titres élevés d'anti-β2GpI et les pertes fœtales étaient associés (OR 5.25 ; IC95 [1.04-27.1]; p=0.041). Vingt-quatre études ont été sélectionnées suite à la revue systématique de la littérature, représentant une population de 2992 patients sclérodermiques. La prévalence des aPL dans cette population était très variable (entre 0 et 58%). La manifestation clinique associée à la présence d'aPL la plus fréquente était l'HTAP.

Conclusion. Cette étude retrouve une prévalence des aPL dans la ScS de 6.4% (IC95 [3.4-9.5]). La présence d'aPL était associée aux TV et aux pertes fœtales. Ces données apportent des éléments supplémentaires concernant l'atteinte vasculaire de la ScS.

Composition du Jury :

Président : Professeur Pierre-Yves Hatron

Assesseurs : Professeur Eric Hachulla, Professeur David Launay, Professeur Marc Lambert, Docteur Sylvain Dubucquoi, Docteur Vincent Sobanski (Directeur de Thèse)