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ORIGINAL ARTICLE

Anti-RNP/Sm antibodies in patients with systemic lupus erythematosus and its role in thrombosis: a case-control study

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Abstract

Objective To validate the association of thrombotic events with positive lupus anticoagulant (LA) and co-presence of anti-RNP/Sm, as well as the diagnostic accuracy of this combination of antibodies for thrombosis.

Methods Case-control study of patients with systemic lupus erythematosus (SLE) who presented thrombosis after SLE diagnosis and controls with SLE without thrombosis. Comorbidities, traditional risk factors, clinical variables, and treatment were evaluated. Antiphospholipid (aPL) and anti-RNP/Sm antibodies were determined.

Results Sixty-three cases and 63 controls were studied, 88% women, median age of 40 years, and disease duration of 135 months at study inclusion. No differences were found between groups regarding age, comorbidities, or clinical characteristics at SLE diagnosis. Patients with thrombosis were more frequently positive for anti-RNP/Sm ($p = 0.001$), IgG aCL ($p = 0.02$), IgG anti-B2GPI ($p = 0.02$), IgM anti-B2GPI ($p = 0.02$), LA ($p < 0.001$), the combination of anti-RNP/Sm + LA ($p < 0.001$), and aPL triple marker ($p = 0.002$), compared to controls. The combination of anti-RNP/Sm + LA, SLEDAI-2 K, and prednisone dose was associated with thrombosis ($p < 0.05$). The combination of anti-RNP/Sm + LA showed 56% sensitivity, 79% specificity, 73% positive predictive value, 64% negative predictive value, positive likelihood ratio (LR) 2.69, and negative LR 0.56 for predicting thrombosis. No difference was found in the comparison of area under the curve between LA alone and the combination of anti-RNP/Sm + LA ($p = 0.73$).

Conclusion Thrombosis was associated with disease activity, dose of prednisone, and the combination of anti-RNP/Sm antibodies and LA. This combination of antibodies could be useful in the identification of SLE patients at risk of thrombosis.

Keywords Diagnostic accuracy · Lupus anticoagulant · Anti-RNP/Sm · Systemic lupus erythematosus · Thrombosis

Introduction

Thrombosis is an important cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE) and occurs with greater frequency and at a younger age than in the general population [1]. It has been reported in 7.2–12% of patients with SLE and accounts for 26% of mortality, similar to active SLE and infections [2, 3].

Ethnicity, disease duration, and type of thrombotic event (venous, VTE or arterial, ATE) influence the incidence of thrombosis in SLE patients. Mean annual incidence of VTE in a cohort of 516 Chinese patients with SLE was 4.2/1000 patient-years [4], while 9% of 570 patients from the multiethnic LUMINA cohort (lupus in minorities, nature versus nurture) with ≤ 5-year disease duration developed at least 1 VTE after SLE diagnosis [5]. Thrombosis occurred in 16% (11% ATE and 5% VTE) of 544 patients with SLE of recent onset (< 1 year) during a follow-up period of 6.3 years, in particular during the first 5 years after SLE diagnosis [2]. Moreover, incidence of arterial and venous thrombosis in a prospective multiethnic cohort of 625 patients with recent-onset SLE was

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16 and 13/1000 patient-years, respectively, with differences in the cumulative risk of ATE and VTE during follow-up among Chinese, Afro-Americans, and Caucasians [6].

Mechanisms mediating thrombosis in SLE are diverse and include both intrinsic and disease-independent factors. Inflammation, atherosclerosis and endothelial damage, as well as traditional risk factors such as male gender, hyperhomocysteinemia, smoking, dyslipidemia, arterial hypertension, and venous insufficiency are associated with risk of thrombosis [1, 7–10]. Disease-related risk factors for thrombosis include age at onset; disease duration and activity; prednisone dose; use of immunomodulating medication; and cutaneous vasculitis, nephrotic syndrome, and antiphospholipid (aPL) antibodies [1, 5, 8, 11].

Positivity for aPL antibodies is present in 40–60% of SLE patients and is associated with increased risk of thrombosis due mainly to resistance to natural anticoagulants such as protein C, impaired fibrinolysis, activation of endothelial cells to a pro-coagulant phenotype, and activation of platelets [12]. The strongest association of these antibodies with thrombosis holds for the persistence in positivity, presence of moderate to high titles, and aPL triple marker positivity [9, 13–17].

Not all SLE patients that are aPL positive develop thrombosis, and conversely, not all the thrombotic events (TE) in these patients can be explained exclusively due to the presence of these antibodies. This suggests the co-existence of several factors with synergic effect on the risk of thrombosis. In a recent study that investigated the risk factors associated with VTE and ATE in an inception cohort of SLE patients, we found that the presence of the combination of lupus anticoagulant (LA) and anti-RNP/Sm antibodies was a risk factor for VTE (OR 6.39, 95% CI 1.37–29.86, $p = 0.02$) [11]. This combination of antibodies showed 25.9% sensitivity (SN), 96.2% specificity (SP), 50% positive predictive value (PPV), 89.8% negative predictive value (NPV), and positive likelihood ratio (LR) 6.0 (95% CI 2.3–15.6), improving the accuracy of LA alone for predicting VTE [11].

Since thrombosis plays a prominent role in SLE affecting morbidity and mortality, we aimed to validate the association of thrombotic events with positive LA and co-presence of anti-RNP/Sm, as well as the diagnostic accuracy of this combination of antibodies for thrombosis in SLE patients.

Materials and methods

After obtaining approval from the Institutional Review Board (approval number 1730), we conducted a case-control study of patients > 18 years old, with SLE diagnosis (≥ 4 American College of Rheumatology revised and updated classification criteria) [18, 19], who presented at least one thrombotic event after SLE diagnosis (cases). Controls were patients with SLE without history of thrombosis, paired 1:1 with cases according to age, gender, and disease duration at the moment of study inclusion. All patients provided written informed consent according to the Declaration of Helsinki and were attended at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, a tertiary care center in Mexico City. Patients with thrombotic events or receiving anticoagulant therapy prior to SLE diagnosis, those with diagnosis of cancer or known hereditary hypercoagulability syndromes, and those with overlapping autoimmune diseases (including mixed connective tissue disease, MCTD) were excluded.

All data were retrieved from the medical records and included demographic characteristics, anthropometrics, and smoking. Comorbidities considered at SLE diagnosis were diabetes mellitus, hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg) on at least two occasions, heart failure, and dyslipidemia (total serum cholesterol ≥ 200 mg/dl and/or total triglycerides ≥ 150 mg/dl). Clinical variables at SLE diagnosis included age, disease duration, and manifestations according to ACR criteria [18, 19].

Variables assessed at the time of thrombosis were age, disease duration, clinical manifestations including cutaneous vasculitis and nephrotic syndrome (urinary protein exceeding 3.5 g per 1.73 m^2 of body surface area per day). Disease activity was assessed using the SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index) [20], while damage was evaluated using SLICC/ACR Damage Index (Systemic Lupus International Collaborative Clinics/American College of Rheumatology) [21], modified to exclude those variables correlated with TE (i.e., cerebrovascular accident, pulmonary infarction, myocardial infarction, VTE, and intestinal infarction). Other variables associated with thrombosis were immobilization during at least 7 days, recent surgery, and use of hormone replacement therapy or oral contraceptives. We considered medication at the time of thrombosis, including prednisone, immunosuppressants, antimalarials, and aspirin.

Thrombosis was defined as clinical signs and symptoms of vascular occlusion, confirmed by studies as follows, pulmonary thromboembolism was documented with computed tomography (CT), ventilation/perfusion scan or lung biopsy; deep-vein thrombosis, ATE of the extremities, and visceral thrombosis were evaluated with Doppler ultrasound, CT, or angiography; cerebrovascular events with CT and/or magnetic resonance imaging, and myocardial infarction with electrocardiogram, cardiac enzymes and coronarography. VTE included deep-vein thrombosis of the extremities, pulmonary thromboembolism, cerebral, retinal, or visceral. ATE included stroke, myocardial infarction, peripheral, visceral, or retinal.

Peripheral blood samples were obtained at the time of patient recruitment and were stored at − 70 °C. Determination of autoantibodies included anti-Sm and anti-RNP/Sm (IgG isotype) by ELISA (*Orgentec Diagnostika*; *Mainz*, *Germany*); IgG and IgM anti-β2-glycoprotein I (anti-B2GPI), IgG and IgM anticardiolipin (aCL) antibodies

(*Inova Diagnostics*; *San Diego*, *CA*, *USA*), processed according to the commercial manufacturer instructions in a DSX System (DYNEX Technologies). Positivity was considered with regard to the 95th percentile in our healthy population for anti-Sm and anti-RNP/Sm, while the 99th percentile was considered for aCL and anti-B2GPI. Moderate to high titers of aCL and anti-B2GPI were defined as > 40 GPL [22]. Lupus anticoagulant (LA) was processed using two methods: the coagulometric test (LA1 reagent screening/LA2 reagent confirmation, *Siemens*) based on the dilute Russell's viper venom time method (dRVVT), and the silica clotting time test, according to the international recommendations [23, 24].

Statistical analysis

Sample size calculation was performed using the formula for case-control studies, considering the data (proportion exposed and OR) of our previous study [11], 80% power, and a 0.05 significance level of 1.96. The result was $n = 126$ (63 cases and 63 controls). Continuous variables are expressed as mean ± standard deviation (SD) or median with minimum and maximum range; categorical variables as counts and percentages. Differences between groups were evaluated with the Student's *t* test or Mann-Whitney *U* test for continuous variables and Chi-square or Fisher's exact test for categorical variables. Spearman test was used for correlation analysis; univariate logistic regression analyses and multivariate analyses were performed, using logistic regression to analyze associations between significant variables ($p \leq 0.10$) identified from the bivariate analyses and risk for TE. Significant variables had to be present in at least 20% of patients with TE to be eligible to enter the model. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. A value of $p < 0.05$ was set and two-sided values are reported.

For time sensitive variables, i.e., disease activity, damage accrued, treatment, and thrombosis risk factors, a dummy date (random date during follow-up) was calculated for patients without TE. It was calculated considering the time in months elapsed between SLE diagnosis and the date of inclusion to the study using an electronic random number generator (random.org).

We calculated SN, SP, PPV, NPV, positive and negative LR with 95% CI for autoantibodies to predict TE. Receiver operating characteristic (ROC) curves were plotted and the areas under the curve (AUC) were measured and compared to evaluate the diagnostic ability of the autoantibodies in thrombosis.

All analyses were done using Stata (Stata Corp; College Station, Texas USA), version 12.0.

Results

A total of 126 SLE patients were included, 63 with thrombosis (cases), and 63 without thrombosis (controls). Eighty-eight percent were women, with a median (range) age of 40 (18–69) years, and disease duration of 135 (3–523) months at study inclusion. There were no differences in disease duration between patients with and without thrombosis at the time of study inclusion (median of 138 vs 132 months, respectively, $p = 0.46$).

At SLE diagnosis, patients had a median age of 26 (13–60) years. No differences were found between patients with and without thrombosis regarding age, comorbidities (body mass index, smoking, diabetes, dyslipidemia, hypertension, and heart failure), or clinical characteristics at SLE diagnosis (Table 1).

With regard to autoantibodies, patients with thrombosis were more frequently positive for anti-RNP/Sm (83% vs 62%, $p = 0.001$); IgG aCL (29% vs 11%, $p = 0.02$); IgG anti-B2GPI (22% vs 6%, $p = 0.02$); IgM anti-B2GPI (19% vs 5%, $p = 0.02$); LA (63% vs 27%, $p < 0.001$); the combination of anti-RNP/Sm + LA (56% vs 21%, $p < 0.001$); and aPL triple marker (19% vs 2%, $p = 0.002$), compared to patients without thrombosis (Table 2).

At the time of thrombosis/dummy date, cases showed shorter disease duration (37 vs 57 months, $p = 0.002$); higher disease activity (SLEDAI-2 K of 4 vs 0, $p < 0.001$); more frequency of immobilization (10% vs 0, $p = 0.002$), and use of prednisone (78% vs 48%, $p = 0.001$), compared to controls. Median daily dose of prednisone was higher in patients with thrombosis (15 mg vs 7.5 mg, $p = 0.0004$), and more patients without thrombosis were receiving methotrexate (13% vs 2%, $p = 0.03$). No differences were found between groups in other traditional risk factors for thrombosis, damage accrued, or clinical manifestations such as cutaneous vasculitis and nephrotic syndrome (Table 3).

Univariate logistic regression analysis showed the following variables associated with thrombosis: aPL triple marker (OR 14.58, 95% CI 1.83–115.99, $p = 0.01$); anti-B2GP1 any isotype (OR 5.39, 95% CI 1.87–15.51, $p = 0.002$); the combination of anti-RNP/Sm + LA (OR 4.8, 95% CI 2.18–10.56, $p < 0.001$); LA (OR 4.7, 95% CI 2.2–10.02, $p < 0.001$); use of prednisone (OR 3.85, 95% CI 1.77–8.33, $p = 0.001$); IgG aCL (OR 3.19, 95% CI 1.22–8.33, $p = 0.01$); anti-RNP/Sm (OR 2.9, 95% CI 1.27–6.64, $p = 0.01$); SLEDAI-2K (OR 1.22, 95% CI 1.09–1.35, $p < 0.001$); and prednisone dose (OR 1.06, 95% CI 1.01–1.11, $p = 0.004$). No correlation was found between disease activity determined by SLEDAI-2K at the time of thrombosis and the positivity for anti-RNP/Sm + LA ($r = 0.15$, $p = 0.08$).

In the multivariate analysis, we found that the combination of anti-RNP/Sm + LA (OR 5.98, 95% CI 2.17–16.47, $p = 0.001$), SLEDAI-2K (OR 1.18, 95% CI 1.04–1.32, $p = 0.007$), and prednisone dose (OR 1.08, 95% CI 1.03–1.12, $p < 0.001$) was associated with thrombosis.

Two-thirds of the thrombotic events (43 patients, 68%) were VTE, being deep venous thrombosis the most frequent

Table 1 Demographic, clinical characteristics and comorbidities at SLE diagnosis

Variables	All $N = 126$	Thrombosis $N = 63$	No thrombosis $N = 63$	p^c
Female—*n* (%)	111 (88)	55 (87)	56 (89)	1.00
Age at diagnosis—years[a]	26 (13–60)	26 (13–60)	26 (13–56)	0.96
Disease duration—months[a]	135 (3–523)	138 (3–523)	132 (11–456)	0.46
Comorbidities				
BMI[b]	25.7 ± 5.6	26.3 ± 5.7	25.2 ± 5.4	0.24
Smoking—*n* (%)	8 (6)	5 (8)	3 (5)	0.71
Diabetes—*n* (%)	4 (3)	1 (2)	3 (5)	0.61
Dyslipidemia—*n* (%)	22 (17)	11 (17)	11 (17)	1.00
Hypertension—*n* (%)	25 (20)	15 (24)	10 (16)	0.37
Heart failure—*n* (%)	2 (2)	0	2 (3)	0.49
Clinical characteristics				
Number of criteria at diagnosis[a]	5 (4–8)	5 (4–8)	4 (4–7)	0.06
Photosensitivity—*n* (%)	25 (20)	12 (19)	13 (21)	1.00
Malar rash—*n* (%)	41 (33)	23 (37)	18 (29)	0.44
Oral ulcers—*n* (%)	44 (35)	24 (38)	20 (32)	0.57
Discoid lupus—*n* (%)	8 (6)	1 (2)	7 (11)	0.06
Arthritis—*n* (%)	94 (75)	47 (75)	47 (75)	1.00
Serositis—*n* (%)	41 (33)	22 (35)	19 (30)	0.70
Renal disorder—*n* (%)	59 (47)	25 (40)	34 (54)	0.15
Neurological disorder—*n* (%)	7 (6)	4 (6)	3 (5)	1.00
Hematologic disorder—*n* (%)	79 (63)	43 (68)	36 (57)	0.26
Immunologic disorder—*n* (%)	95 (75)	52 (83)	43 (68)	0.09
ANA—*n* (%)	107 (85)	57 (90)	50 (79)	0.13
Obstetric APS—*n* (%)	6 (5)	4 (6)	2 (3)	0.68

BMI body mass index, *ANA* antinuclear antibodies, *APS* antiphospholipid syndrome

[a] Median (min-max)

[b] Mean ± SD

[c] Comparison between patients with and without thrombosis

in 19 patients (30%). Pulmonary embolism occurred in eight (13%), five patients experienced simultaneously deep venous thrombosis and pulmonary embolism, and the rest of the VTE were retinal vein in five (8%), cerebral venous sinus in three (5%), and visceral thrombosis in three (5%). ATE occurred in 20 patients (32%), being cerebrovascular events and transient ischemic attack the most frequent in 16 (25%), while myocardial infraction occurred in 3 (5%), and visceral arterial thrombosis in 1 (2%). Prior to the thrombotic event, 11 patients were receiving antiplatelet drugs, and 2 patients received prophylactic anticoagulation. After thrombosis, 89% of patients received total anticoagulation with vitamin K antagonists. No differences were found in patients with VTE and ATE in the positivity for the combination of anti-RNP/Sm + LA (56% vs 45%, $p = 0.58$).

The combination of anti-RNP/Sm + LA showed 56% SN, 79% SP, 73% PPV, 64% NPV, positive LR 2.69 (95% CI 1.58–4.45), and negative LR 0.56 (95% CI 0.41–0.76) for predicting thrombosis. Table 4 shows the diagnostic accuracy of antibodies in thrombosis.

LA alone showed the best AUC for thrombosis (0.68), almost identical to the combination of anti-RNP/Sm + LA (0.67). For anti-RNP/Sm alone, it was 0.60, and aPL triple marker 0.58. No difference was found in the comparison of AUC between LA alone and the combination of anti-RNP/Sm + LA (Bonferroni $p = 0.73$), although a difference was seen in the comparison of this combination with aPL triple marker (Bonferroni $p = 0.04$). Figure 1 displays the ROC curves for antibodies in thrombosis.

Discussion

This analysis of 126 SLE patients, mostly women, with and without thrombosis, found no differences in comorbidities or clinical characteristics among groups. Traditional risk factors for thrombosis showed a low prevalence (< 25%); TE were mainly venous and occurred at a median of 3 years since SLE diagnosis. Variables associated with thrombosis were the combination of anti-RNP/Sm + LA, disease activity, and dose of

Table 2 Serologic characteristics

Variables	All $N = 126$	Thrombosis $N = 63$	No thrombosis $N = 63$	p[a]
Anti-RNP/Sm—*n* (%)	91 (72)	52 (83)	39 (62)	*0.001*
Anti-Sm—*n* (%)	91 (72)	49 (78)	42 (67)	0.23
IgG aCL—*n* (%)	25 (20)	18 (29)	7 (11)	*0.02*
IgG aCL moderate to high titers—*n* (%)	12 (10)	9 (14)	3 (5)	0.12
IgM aCL—*n* (%)	15 (12)	11 (17)	4 (6)	0.09
IgM aCL moderate to high titers—*n* (%)	5 (4)	3 (5)	2 (3)	1.00
Any aCL—*n* (%)	32 (25)	24 (38)	8 (13)	*0.002*
IgG anti-B2GPI—*n* (%)	18 (14)	14 (22)	4 (6)	*0.02*
IgG anti-B2GPI moderate to high titers—*n* (%)	5 (4)	3 (5)	2 (3)	1.00
IgM anti-B2GPI—*n* (%)	15 (12)	12 (19)	3 (5)	*0.02*
IgM anti-B2GPI moderate to high titers—*n* (%)	7 (6)	5 (8)	2 (3)	0.44
Any anti-B2GPI—*n* (%)	25 (20)	20 (32)	5 (8)	*0.001*
LA—*n* (%)	57 (45)	40 (63)	17 (27)	*< 0.001*
Anti-RNP/Sm + LA—*n* (%)	48 (38)	35 (56)	13 (21)	*< 0.001*
aPL double marker positivity—*n* (%)	18 (14)	11 (17)	7 (11)	0.44
aPL triple marker positivity—*n* (%)	13 (10)	12 (19)	1 (2)	*0.002*

aCL anticardiolipin, *anti-B2GPI* anti-β2-glycoprotein I, *LA* lupus anticoagulant, *aPL* antiphospholipid

[a] Comparison between patients with and without thrombosis. Italicized values represent significant p values

prednisone. No association was found between traditional risk factors or specific SLE clinical manifestations and thrombosis.

The most frequent individual antibody present in patients with thrombosis was anti-RNP/Sm (83%), followed by LA

Table 3 Systemic lupus erythematosus characteristics at thrombosis/dummy date

Variables	All $N = 126$	Thrombosis $N = 63$	No thrombosis $N = 63$	p[b]
Age—years[a]	33 (15–65)	32 (15–65)	34 (19–60)	0.43
Disease duration—months[a]	48 (0–311)	37 (0–311)	57 (1–309)	*0.002*
SLEDAI-2K score[a]	2 (0–31)	4 (0–31)	0 (0–14)	*< 0.001*
SLICC/ACR Damage Index, modified[a]	0 (0–4)	0 (0–4)	0 (0–3)	0.51
Surgery—*n* (%)	2 (2)	1 (2)	1 (2)	1.00
Immobilization—*n* (%)	6 (5)	6 (10)	0	*0.002*
Oral contraceptives—*n* (%)	1 (1)	1 (2)	0	1.00
Replacement hormone therapy—*n* (%)	1(1)	0	1 (2)	1.00
Nephrotic syndrome—*n* (%)	11 (9)	8 (13)	3 (5)	0.20
Vasculitis—*n* (%)	3 (2)	3 (2)	0	0.24
Prednisone—*n* (%)	79 (63)	49 (78)	30 (48)	*0.001*
Dose of prednisone—mg[a]	10 (1–100)	15 (1–100)	7.5 (2.5–52.5)	*0.0004*
Antimalarials—*n* (%)	61 (48)	25 (40)	36 (58)	0.07
Azathioprine—*n* (%)	48 (38)	24 (38)	24 (38)	1.00
Cyclophosphamide—*n* (%)	11 (9)	6 (10)	5 (8)	1.00
Mycophenolate mofetil—*n* (%)	16 (13)	5 (8)	11 (17)	0.18
Methotrexate—*n* (%)	9 (7)	1 (2)	8 (13)	*0.03*
Aspirin—*n* (%)	21 (17)	11 (17)	10 (16)	1.00

SLEDAI-2K Systemic Lupus Erythematosus Disease Activity Index, *SLICC/ACR* Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index

[a] Median (min-max)

[b] Comparison between patients with and without thrombosis. Italicized values represent significant p values

Table 4 Accuracy of antibodies for predicting thrombosis

Tests	SN (%)	SP (%)	PPV (%)	NPV (%)	LR+ (95% CI)	LR− (95% CI)
Anti-RNP/Sm	83	38	57	69	1.33 (1.07–1.67)	0.46 (0.25–0.85)
LA	63	73	70	67	2.35 (1.50–3.68)	0.50 (0.35–0.72)
aPL double marker	17	89	61	52	1.57 (0.65–3.79)	0.93 (0.80–1.07)
aPL triple marker	19	98	92	55	12 (1.61–89.55)	0.82 (0.73–0.93)
Anti-RNP/Sm + LA	56	79	73	64	2.69 (1.58–4.58)	0.56 (0.41–0.76)

LA lupus anticoagulant, *aPL* antiphospholipid, *SN* sensitivity, *SP* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio

(63%); therefore, the combination of anti-RNP/Sm + LA antibodies was the most frequent in 56% of patients with thrombosis, compared to aPL double and triple marker, present in only 17% and 19%, respectively.

Disease activity, prednisone dose, and LA have previously been identified as risk factors for thrombosis in SLE patients [1, 5, 8, 11]. The finding of positivity for anti-RNP/Sm in a great proportion of our patients with thrombosis, overcoming in frequency other traditionally known antibodies associated with thrombosis such as aCL, focused our attention in the possible role of this antibody in combination with LA as a risk factor for thrombosis. Interestingly, no differences were found in the titles of anti-RNP/Sm between patients with and without thrombosis, and the role of the antibody driving the immune response at disease onset was also ruled out since patients had median disease duration of 11 years at the time that the antibodies were determined. Moreover, no correlation was found between disease activity at the time of thrombosis and the presence of anti-RNP/Sm + LA, suggesting an independent role of these antibodies in thrombosis. The diagnostic accuracy of the combination of anti-RNP/Sm + LA for thrombosis showed higher SP and positive LR compared to LA individually, but not superior to aPL triple marker.

Antibodies against U1-ribonuclear protein (U1-RNP or RNP/Sm) are characteristically associated with MCTD, although their presence is also common in SLE and systemic sclerosis, with differences in clinical features and uncertain role in disease pathogenesis. These antibodies are present in 26% of SLE patients prior to the onset of clinical illness and frequently appear within 1 year of diagnosis. Furthermore, anti-U1-RNP antibodies act as anti-endothelial cell antibodies (AECA) and interact with mononuclear and endothelial cells to mediate tissue injury and vasculopathy in connective tissue disease by increasing the production of IL-1 and IL-6 and up-regulating the expression of intracellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and class II major histocompatibility complex (MHC) molecules [25, 26].

Clinical and immunological phenotypes associated with the presence of anti-RNP/Sm differ according to ethnicity

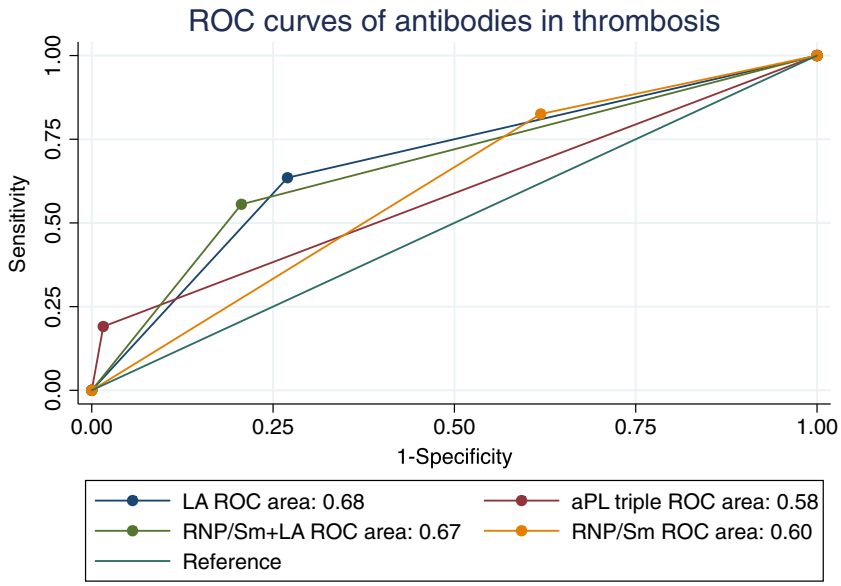

Fig. 1 ROC curves for antibodies in thrombosis. LA, lupus anticoagulant; aPL, antiphospholipid

and to the diagnosis of SLE or MCTD, even though great proportion of patients anti-RNP/Sm positive that meet criteria for MCTD also meet criteria for SLE. For example, patients with MCTD and positive or high titer anti-RNP/Sm show lower rates of diffuse proliferative glomerulonephritis or severe central nervous system manifestations; conversely, they show higher rates of Raynaud's phenomenon, and trends towards increased B cell activation [27, 28]. Moreover, anti-RNP/Sm antibodies are more prevalent in Afro-Caribbean patients with SLE (53%), compared to Europeans (20%), and Asians (29%), and are associated with clinical manifestations such as rash, alopecia, mouth ulcers, serositis, neurological, joint, and renal involvement [29]. These findings suggest that the presence of anti-RNP/Sm antibodies could possess prognostic value [28], and their multiple effects on the innate and adaptive immune responses implicate them in autoimmunity, inflammation, and tissue damage.

Possible mechanisms to explain the association of anti-U1-RNP antibodies with thrombosis are the recognition of a variety of antigens on the endothelial surface of the pulmonary arteries, including the components of U1-RNP or other unknown polypeptides that may be one of the triggers of endothelial cell inflammation in patients with connective tissue diseases [30]. Furthermore, anti-U1-RNP act as AECA, and pathophysiological effects of AECA include the activation of endothelial cells, resulting in increased leucocyte adhesiveness to endothelial surfaces, cytokine production, activation of coagulation, vascular injury, and thrombosis [31]. Endothelial cell activation has also been described as one of the mechanisms mediating thrombosis in APS, by favoring the appearance of a procoagulant endothelial phenotype [12, 32].

Another mechanism mediating thrombosis in SLE involves neutrophil extracellular traps (NETs) release, a form of neutrophil cell death that results in the externalization of decondensed chromatin decorated with granular and nuclear proteins. NETs are an integral component of thrombi, serve as structural scaffolding for entrapment and aggregation of platelets and erythrocytes, release proteases that activate the coagulation cascade, and damage the endothelium [33, 34]. Some patients with SLE have a deficiency in circulating DNase function and therefore, an impaired ability to degrade NETs in plasma, resulting in increased levels of circulating NETs. Circulating antibodies such as anti-RNP and anti-LL-37 in SLE patients stimulate neutrophils to undergo NETosis [34–37]. NETs in SLE exert immunostimulatory effects, promote the production of type I interferons by plasmocytoid dendritic cells, and have a direct role in endothelial damage [34]. A positive correlation between circulating levels of NETs and aPL antibodies in patients with primary APS has been found, suggesting that circulating NETs contribute to thrombosis in these patients [33]. Furthermore, a murine model of thrombosis supported an in vivo role of NETs in aPL-mediated venous thrombosis [38].

Individual autoantibodies are associated with clinical features in autoimmune diseases, while clusters of autoantibodies have been described to differentiate between disease subsets with prognostic implications. A study of 1357 SLE patients from a prospective longitudinal cohort investigated autoantibody clusters and their associations with clinical features and damage accrual [39]. Cluster 1 (anti-Sm, anti-RNP, $n = 451$), cluster 2 (anti-dsDNA, anti-Ro, anti-La, $n = 470$), and cluster 3 (anti-dsDNA, LA, aCL, $n = 436$) were defined. In cluster 1, frequency of LA positivity was 14%; prevalence of cerebrovascular accident was 6%, myocardial infraction 3%, and venous thrombosis 2%, significantly lower (except for myocardial infraction) than in cluster 3 (13%, 5%, and 8%, respectively). Risk of thrombosis is therefore influenced (among other factors) by clusters of autoantibodies; the number, titles, and persistence of positive aPL antibodies (single, double, or triple positivity), as well as coexistence of autoimmune disease [15, 40].

Our study has some limitations. First, as a result of the cross-sectional design, assessment of antibodies occurred at time of enrollment and not at necessarily at the time of thrombosis; therefore, persistence in the positivity during follow-up, causality, and predictive value of these antibodies in thrombosis could not be evaluated. Nevertheless, the findings of our previous study suggest that in SLE patients, the combination of anti-RNP/Sm + LA is also present before and at the time of thrombosis [11]. Second, as a result of patient selection, 89% of patients with thrombosis were receiving oral anticoagulants at the time of LA determination, although only 30 patients showed a therapeutic international normalized ratio (INR) at the time of the sample collection. In this context, frequency of LA could have been overestimated and this could possibly explain its high prevalence and a better diagnostic accuracy. The detection of anti-phosphatidylserine/prothrombin antibodies may offer additional information, as emerging evidence supports the clinical utility of these antibodies in establishing the risk of thrombosis and other manifestations of APS in patients with SLE and other systemic autoimmune diseases [41, 42].

The strengths of our study include the approach of thrombosis considering traditional risk factors, disease-associated variables, and autoantibodies, as well as the inclusion of cases and controls according to sample size calculation to validate our previous findings.

In our study of SLE patients, thrombosis was associated with disease activity, dose of prednisone, and the combination of anti-RNP/Sm antibodies and LA. This combination of antibodies could be useful in the identification of SLE patients at risk of thrombosis, although its clinical utility does not seem to be superior to LA individually or aPL triple marker. Future studies should explore the pathogenic effect of anti-RNP/Sm antibodies alone or in combination with LA in the setting of thrombosis either in patients with SLE or in murine models of thrombosis.

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Authors' contributions MCZM, AHA, and JRD designed the study; MCZM, CANA, and AGVR participated in acquisition of data; AHA and JRD analyzed and interpreted data; MCZM, AHA, and JRD drafted the manuscript; MCZM, AHA, JRD, CANA, and AGVR revised the manuscript.

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Compliance with ethical standards Research was conducted in compliance with the Helsinki Declaration. Informed consent and approval by the local ethical committee was obtained.

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