Descriptive analysis of patients positive for anti-phospholipid antibodies included in two Italian registries

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ABSTRACT

The definite diagnosis of antiphospholipid syndrome (APS) depends on the laboratory performance and clinicians' interpretation. Results from two Italian inception cohort studies of antiphospholipid antibodies (aPL)-positive subjects, the Italian Survey on ANtiphoSpholipid antibody Positive Individuals Registry (INSPIRE) and the Survey on AnTicoagulated Patients- RegisTry (START) have been compared. Data from INSPIRE were collected by rheumatologists of the FIRMA group while those of START by physicians working in Italian thrombosis centers. Evidence on several, still unraveled, clinical and methodological aspects of 'real life' aPL testing and APS diagnosis was collected. In this paper, we report the characteristics of 123 cases enrolled in INSPIRE and 229 in START registries, with particular reference to the reasons why these tests were requested, the obtained aPL profile, and consequent treatment. Laboratory testing for aPL in the absence of clinical criteria defining APS was more frequent in INSPIRE ($p<0.0001$). The rate of patients in classification category I (more than one aPL test positive) was significantly higher in START ($p<0.0001$) as well as the use of warfarin. A wide variety of treatments has been observed in patients in classification category II (single aPL positivity). These data indicate that there is a need to harmonize many aspects among the various specialists dealing with APS.

Introduction

Anti-phospholipid syndrome (APS) is the most studied form of acquired thrombophilia. It is defined as the concomitant presence of thromboembolic events or pregnancy morbidity in patients with persistently positive circulating anti-phospholipid antibodies (aPL).[1] Besides thrombosis and pregnancy morbidity, other clinical manifestations affecting every organ are often associated with

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Citation: Pengo V, Sarti L, Antonucci E, et al Descriptive analysis of patients positive for anti-phospholipid antibodies included in two Italian registries. Bleeding, Thrombosis, and Vascular Biology 2023;2:92.

Key words: antiphospholipid syndrome; registries; classification.

Contributions: VP, substantial contribution to concept and design of the study and writing of the first draft of the manuscript; LS, EA, SDA, EB, GD, contributed to analyze and interpret the data; DP, PLM, RG, AR, GP, substantial contribution to collect data, critical writing and revising the intellectual content. All the authors approved the final version to be published.

Acknowledgments: FIRMA collaborators: Cristiano Alessandri, Roma-Università La Sapienza-Reumatologia 31; Orietta Borghi, Roberta Gualtierotti Pierluigi Meroni, Milano-Ospedale Auxologico-IRL 29; Elena Bartoloni Bocci, Onelia Bistoni, Giacomo Cafaro, Carlo Perricone, Alessia Alunno, Perugia-Università Medicina 18; Federica Zunino, Immunologia, Bergamo 16; Laura Andreoli, Angela Tincani, Reumatologia, Brescia 14; Annamaria Iuliano, Giandomenico Sebastiani, Reumatologia, Ospedale San Camillo Roma-10; Arianna Damiani, Maurizio Benucci, Maria Infantino, Ospedale San Giovanni di Dio, Firenze 3; Amelia Rigon, Elisabetta Cerchiara Reumatologia Campus Bio-medico-Roma 2; START-antiphospholipid register collaborators: Poli Daniela, Lotti Elena, Centro Trombosi Firenze (FI) 60; Sivera Piera, Ematologia-Mauriziano Torino (TO) 29; Barcellona Doris, Centro Emocoagulopatie Monserrato (CA) 22; Prisco Domenico, Canfora Maria, SODC Medicina Interna, Firenze 15; Sarti Luca, Medicina Interna D'urgenza-Modena 14; Visonà Adriana, Medicina Vascolare, Castelfranco Veneto 13; Chistolini Antonio, Sez Ematologia, Policlinico Umberto I, Roma (RM) 9; De Candia Erica, Università Cattolica del Sacro Cuore, Roma (RM) 8; Falanga Anna, Centro emostasi e trombosi, Bergamo (BG) 8; Bucherini Eugenio, Amb. anticoagulanti Faenza - AUSL di Ravenna (RA) 7; Tufano Antonella, UOC Med Interna, Napoli (NA) 7;Pizzini Attilia, UOC Medicina A-OM-Bologna Bologna 6; Oriana Vincenzo, Centro Emofilia – Reggio Calabria 5; Cotelli Maria Sophia, SSD Neurologia-ASST Valcamonica, Esine (BS) 5; Gresele Paolo, MIV Perugia (PG) 4; Toma Andrea, UOC di Patologia Clinica, Arzignano (VI) 4; Fregoni Vittorio, U.O.C. Medicina Generale, Sondalo (SO) 3; Ageno Walter, Medicina d'Urgenza, Varese (VA) 2; Pedrini Simona, Servizio di laboratorio-Fond. Poliambulanza Brescia (BS) 2; Lodigiani Corrado, Centro Trombosi, Istituto Clinico Humanitas, Milano 2; Bison Elisa, Laboratorio di ricerca sulla Trombosi, Padova 1; Spataro Carmine, UOSD SIMT-Treviglio, Treviglio (BG) 1; Hoxha Ariela, UOC-Med generale 1 Vicenza Vicenza (VI) 1; Banov Laura, UOC Oncoematologia -IRCCS Gaslini Genova (GE) 1.

Conflict of interest: VP received lecture fees from Werfen Group. The other authors declare no potential conflict of interest.

Funding: none.

Ethical approval and consent to participate: APS patients after signing an informed consent were screened for possible participation in the registries.

Availability of data and material: data are available upon reasonable request.

Informed consent: all patients enrolled in the study read and signed the informed consent.

Received: 13 July 2023.
Accepted: 20 September 2023.

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Bleeding, Thrombosis and Vascular Biology 2023; 2:92
doi:10.4081/btvb.2023.92

APS.[2] Therefore, clinicians often prescribe testing for aPL in the presence of signs or symptoms unrelated to the classical manifestations of the syndrome.[3,4] Epidemiological and methodological data on testing for aPL in general and specific laboratories are sparse. Reasons for aPL checking are relevant for the appropriateness, cost-effectiveness, and difficult interpretation of positive results. In the present report, we evaluate the relation between the reasons why aPL was tested, the obtained aPL profile, and the treatment. We explored data from two Italian registries, the INSPIRE which is mainly driven by rheumatologists, and the START which involves thrombosis centers led by hematologists, internists, or clinical pathologists. Differences in the type of patients enrolled, and the aPL profiles are examined. The implications on the interpretation of abnormal test results and the approach to therapeutic interventions in the two registries are discussed.

Materials and Methods

The Interdisciplinary Forum for the Research on Autoimmune Diseases (FIRMA), the Italian branch of the European Autoimmunity Standardization Initiative (EASI) founded in the 90s and devoted to standardizing serology in rheumatic diseases, promoted the Italian Survey on ANtiphoSpholipid antibody Positive Individuals Register (INSPIRE), an Italian registry of aPL-positive individuals. This is an educational program aimed to harmonize the way aPL are detected and interpreted. The Survey on AnTicoagulated Patients- RegisTry (START) aPL register is a prospective register of long-term collected data from Centers for the Diagnosis of Thrombosis and surveillance of antithrombotic therapies promoted by Arianna Foundation on Anticoagulation. Subjects were recruited when testing positive for one or more criteria aPL tests [anti-cardiolipin (aCL) and anti-ß2-Glycoprotein I (anti-ß2GPI) antibodies IgG/IgM at any titer, lupus anticoagulant (LA)] and testing was repeated after 12 weeks. Demographic and clinical data (thrombosis, pregnancy complications, non-criteria clinical manifestations, systemic autoimmune disease, cardiovascular risk factors and treatment) were entered in a web-based case report form. All patients enrolled in the study read and signed the informed consent. Reasons for testing were grouped according to clinical events that defined or did not define APS (thrombosis and pregnancy morbidity) and aPL profiles were classified as category I (more than one aPL test positive) or category II (only one aPL test positive), according to Miyakis.[1]

Results

In total, 123 patients were recruited in the INSPIRE and 229 in the START registries during a 2-year period. Patients' characteristics are summarized in Table 1.

Patients were older in START and female gender was more frequent in both cohorts, although it was higher in INSPIRE. Cardiovascular risk factors and associated autoimmune diseases were more frequent in INSPIRE registry. Antithrombotic treatment differed importantly in the two registries since warfarin use was by far greater in the START registry, which collected data in thrombosis centers.

In 54 of 123 cases (44%) in INSPIRE and in 155 of 229 cases (67%) in START, clinical criteria matched those required in the classification criteria for the diagnosis of APS (Table 2). A higher rate of venous thromboembolism was recorded in the START compared to the INSPIRE registry (p<0.0001). Conversely, clinical criteria not included in the guidelines were more frequent in INSPIRE (p<0.0001). The large majority of specialists who requested aPL testing are rheumatologists in INSPIRE while hematologists, internists and clinical pathologists were the more represented specialists in START registry. Reported reasons for

Table 1. Characteristics of enrolled individuals.

	INSPIRE (n=123)	START (n=229)	P
Age (yrs)	49.7±14.1	54.3±16.9	0.01
Sex (F)	93 (76)	145 (63)	0.02
BMI	25.1±5.2	25.8±6.8	-
Ethnicity			-
Caucasian	121 (98)	223 (97)	-
Asian	1	6	-
Hispanic	1	0	-
Hypertension	37 (30)	77 (34)	-
Diabetes	12 (10)	19 (8)	-
Dyslipidemia	36 (29)	31 (13)	<0.01
Smoking habit	35 (28)	37 (16)	<0.01
Systemic autoimmune diseases	36 (29)	28 (12)	<0.01
Systemic lupus erythematosus	12	24	-
Undifferentiated connective tissue disease	8	-	-
Lupus-like	3	-	-
Rheumatoid arthritis	2	-	-
Sjögren's syndrome	1	3	-
Connective tissue disease	1	-	-
Other	9	-	-

Data are expressed as mean ± standard deviation or n (%). BMI, body mass index.

testing in the absence of clinical criteria defining APS are reported in Table 3 in descending order. Programmed pregnancy, autoimmune diseases, and thrombocytopenia were among the most cited clinical reasons.

Classification of patients in the registries

Among the 123 subjects enrolled in INSPIRE, the request for aPL testing is motivated in 54 cases (43.9%) by clinical criteria of APS. In 27 (50%) of APS patients the aPL profile falls in classification category I (more than one positive test among LAC, IgG/IgM aCL and aβ2GPI), and in the remaining 27 cases in category II (single positive test). The distribution of specific aPL profiles is shown in Figure 1.

Of the 229 subjects enrolled in the START registry, 155 (68%) had the clinical criteria for the diagnosis of APS. Of these, 125 patients had an aPL profile in classification category I and 30 in category II. The distribution of specific profiles is shown in Figure 1B. There was a significant difference in the distribution of classification categories among the registries (p<0.0001). Indeed, classification category II is more represented in INSPIRE, particularly isolated aCL.

Figure 2 reports the antithrombotic treatment according to clinical criteria in APS patients in classification category I in INSPIRE (A) and START (B). In both registries, most of the patients with venous thromboembolism (VTE) were treated with warfarin (73% and 74%, respectively). The majority of patients with arterial thromboembolism (ATE) in INSPIRE are treated with antiplatelet drugs or a combination of warfarin and aspirin plus clopidogrel in special situations (myocardial infarction), while in START warfarin was the most used drug (in 51% of cases). The difference in the use of warfarin in ATE among registries is not statistically significant (p=0.09).

Figure 3 reports the antithrombotic treatment in patients with clinical criteria for APS and classification category II (only one positive test) in INSPIRE (A) and START (B) registries. In the 27 patients of INSPIRE, isolated LA is present in 3 patients, isolated IgG aCL or isolated IgG aβ2GPI in 16, and isolated IgM aCL or isolated IgM aβ2GPI in 8 patients. The antithrombotic regimen in VTE patients of this group are quite heterogenous (Figure 3A): patients received either warfarin or direct oral anticoagulants (DOACs) and in a few cases other treatment. Patients with ATE took antiplatelet drugs or a combination of warfarin and antiplatelet drugs in special situations. In the 2 cases of obstetric APS, Aspirin and Aspirin plus LMWH were used.

Table 2. Reasons for anti-phospholipid antibodies testing clinical criteria.

Clinical criteria	INSPIRE (n=123)	START (n=229)	P
Included in the guidelines	54 (43.9)	140 (67.7)	<0.0001
Venous thromboembolism	29 (23.6)	96 (41.9)	-
Deep vein thrombosis	16	90	
Pulmonary embolism	12	6	
Budd Chiari syndrome	1	-	
Arterial thromboembolism	21 (17.1)	44 (19.2)	-
Transient ischemic attack	8	6	
Ischemic stroke	6	32	
Acute myocardial infarction	3	3	
Unspecified arterial thrombosis	4	3	
Miscarriage	4 (3.2)	15 (6.5)	-
Not included in the guidelines	69 (56.1)	74 (32.3)	<0.0001

Data are expressed as n (%).

Table 3. Patients tested for anti-phospholipid antibodies without the antiphospholipid syndrome clinical criteria in INSPIRE.

		Reasons for testing	Treatment
Laboratory classification category I	Triple+ n=17 IgG n=13 IgM n=4	8 autoimmune diseases 4 thrombophilia screening 2 thrombocytopenia 2 prolonged aPTT 1 thrombophlebitis	7 aspirin 5 no treatment 4 warfarin (1 SLE, 1 Sjögren, 2 thrombophilia screening) 1 DOAC
	Double+ n=33IgG n=13 IgM n=20	10 autoimmune diseases 4 programmed pregnancy 3 thrombophilia screening 3 thrombocytopenia 9 other 4 unknown	23 aspirin 6 warfarin 4 no treatment
Laboratory classification category II	Single+ n=19 IgG n=8 IgM n=11	11 autoimmune disease 4 thrombophilia screening 2 programmed pregnancy 2 unknown	12 no treatment 6 aspirin 1 DOAC

aPTT, activated partial thromboplastin time; DOAC, direct oral anticoagulants; IgG, immunoglobulin G; IgM, immunoglobulin M; SLE, systemic Lupus Erythematosus.

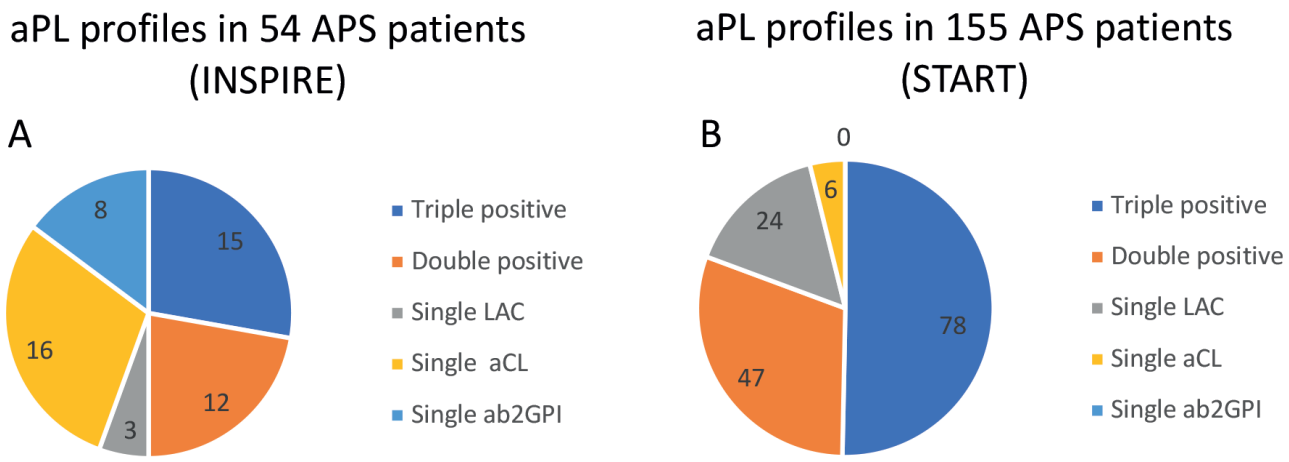

Figure 1. Distribution of anti-phospholipid antibodies profiles among antiphospholipid syndrome patients in INSPIRE (A) and in START (B) registries.

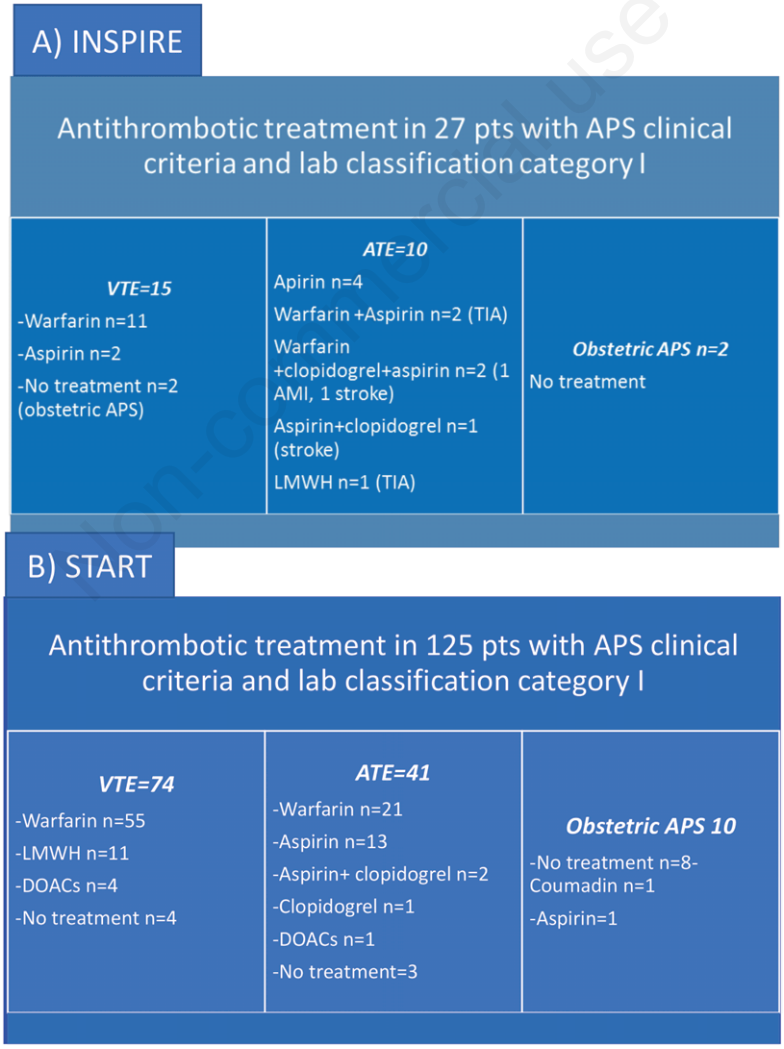

Figure 2. Antithrombotic treatment in patients with antiphospholipid syndrome clinical criteria and laboratory classification category I (more than one anti-phospholipid antibodies positive test) in INSPIRE (A) and START (B) registries.

In the START registry, most patients in classification category II were positive for LA (n=24) and positive for aCL in 6 cases while no patient was positive for isolated aβ2GPI antibodies. In patients with VTE warfarin is more frequently used, but DOACs are also prescribed. In patients with ATE, treatment is highly inhomogeneous with a few patients left without anticoagulant drugs.

Laboratory classification category in patients without anti-phospholipid syndrome clinical criteria (anti-phospholipid antibodies carriers)

Among the 69 individuals tested for aPL without the APS clinical criteria in INSPIRE, 50 are in classification criteria I (17 are triple positive and 33 are double positive). The reasons for testing and antithrombotic treatment are depicted in Table 3. In most cases, specialists prescribe aPL testing in patients with autoimmune diseases (n=29). Despite being aPL carriers (no thromboembolic events or pregnancy morbidity), most of the patients in the laboratory category I receive anticoagulant or antiplatelet therapy. In contrast, most patients in laboratory category II did not receive an antithrombotic treatment. In the 61 patients (data were not available in 13) without clinical criteria in START (Table 4), antiplatelet therapy or no treatment was the choice in classification category I. In contrast, most patients in classification category II did not receive an antithrombotic treatment.

Discussion and Conclusions

This report describes patients positive for aPL antibodies included in the INSPIRE and START registries. The aim of the study was to compare patients' characteristics, the reasons for checking aPL and the obtained aPL profiles with consequent treatment in the setting of rheumatology departments (INSPIRE) and

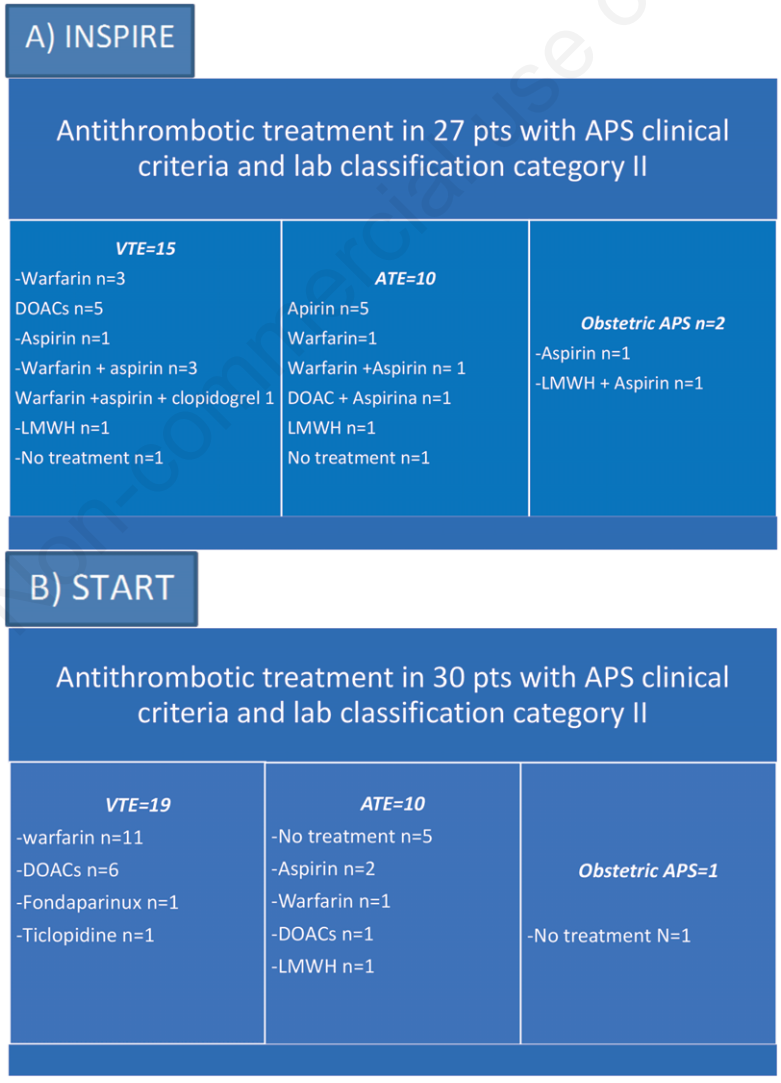

Figure 3. Antithrombotic treatment in patients with antiphospholipid syndrome clinical criteria and laboratory classification category II (only one anti-phospholipid antibodies positive test) in INSPIRE (A) and START (B) registries.

Table 4. Patients tested for anti-phospholipid antibodies without the antiphospholipid syndrome clinical criteria in START.

		Reasons for testing	Treatment
Laboratory classification category I	Triple+ n=19 IgG n=13 IgM n=6	8 autoimmune diseases 4 prolonged aPTT 3 programmed pregnancy 2 superficial thromboflebitis 1 vasculopathy 1 epilepsy	10 aspirin 8 no treatment 1 clopidogrel
	Double+ n=23 IgG n=8 IgM n=15	9 autoimmune diseases 3 thrombophilia screening 2 programmed pregnancy 2 superficial thromboflebitis 1 raynaud phenomenon 3 other 3 unknown	8 aspirin 1 clopidogrel 14 no treatment
Laboratory classification category II	Single+ n=19	6 autoimmune disease 6 prolonged aPTT 3 programmed pregnancy 1 epilepsy 1 migraine 2 unknown	2 LMWH 17 no treatment

aPTT, activated partial thromboplastin time; LMWH, low-molecular-weight heparin; IgG, immunoglobulin G; IgM, immunoglobulin M.

thrombosis centers (START). There was a higher number of carriers (patients without the clinical criteria for APS) in INSPIRE due to aPL testing in a broad way in autoimmune diseases. Indeed, rheumatologists request aPL for patients with autoimmune diseases in the absence of APS clinical criteria as a part of diagnostic work-up and because aPL is considered among the lab criteria for the diagnosis of systemic lupus erythematosus.[5-7] Consequently, signals for the presence of autoantibodies comprising aPL are sweep-searched in autoimmune disorders. This extensive search for aPL in INSPIRE results in completely different aPL profiles from those found in the START register. Isolated positivity (only one positive test) that is not associated with thromboembolic events is more frequent in INSPIRE. In addition, isolated positivity deserves attention as the test may be false-positive or confirmed 12 weeks apart in less than 50% of cases (transient antibodies).[8] In contrast, profiles that are more likely to be associated with thrombosis (positivity in more than one test) are frequent in START where patients with thromboembolic disease are more frequently seen. In that registry, the aPL profile was specifically requested in patients with clinical criteria for APS and in half of them the profile fell in the laboratory classification I (more than one positive test). Of note, many of them show triple or double positivity of IgG isotype, which is believed the aPL profile most often associated with thromboembolic events.[9] In both registries, these patients were accurately treated with warfarin in the case of VTE, while antiplatelet agents were preferred in the case of ATE. Recent guidelines point out the need to treat ATE patients with warfarin or warfarin plus antiplatelet drugs.[10] A few APS patients in the classification category I are treated with DOACs, a practice that is not currently recommended.[10]

More variability in treatment is observed in APS patients in Classification Category II, where both warfarin or DOACs are used in VTE and treatment of ATE is left to the doctor's discretion. Indeed, there are no specific clinical trials addressing this issue. A systematic review by Garcia *et al*.[11] showed that the risk ratio for recurrent VTE after stopping anticoagulant therapy in patients with an anti-cardiolipin antibody was 1.53 (95% CI, 0.76-3.11), and with a LA was 2.83 (95% CI, 0.83-9.64). However, all considered studies had important methodologic limitations. Although a positive aPL test appears to predict an increased risk of recurrence in patients with a first VTE, the strength of this association is uncertain because the available evidence is of very low quality.[11] Finally, the absence of specific clinical trials for aPL carriers also determines the great variety of treatments in both the INSPIRE and START registers. Certainly, the increased demand for aPL testing by rheumatologists may lead them to prescribe antithrombotic treatments outside the standard treatment as there are no *ad hoc* studies. Indeed, apart from SLE patients, in other autoimmune diseases such as systemic scleroderma, Sjogren's syndrome and rheumatoid arthritis the presence of aPL is not associated with an increased risk of thromboembolic events. More studies are needed to determine whether aPL can predispose patients with autoimmune diseases to manifestations of vascular events other than thrombosis, such as renal crisis or pulmonary arterial hypertension.[12]

Data reported herein enhance the vision of contributors and readers leading to a more thoughtful behavior in prescribing and treating aPL-positive patients and hopefully contribute to create a better connection between clinicians and clinical pathologists for the correct interpretation of laboratory results.

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