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Impact of the 2023 ACR/EULAR classification criteria on START2 Antiphospholipid Registry

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Running title: Application of ACR/EULAR classification criteria

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state no conflict of interest. **Research ethics**: The study was conducted according to the ethical principles for medical research as set out in the Declaration of Helsinki. **Informed consent**: All the patients signed an informed consent at the time of enrollment in the Registry. **Permission to reproduce material from other sources**: NA. **Clinical trial registration**: NA. **Author contributions**: VP gave a substantial contribution to concept and design of the study and wrote the first draft of the manuscript. LS, EA, SDA, EB, GD contributed to analyze and interpret the data. DP, GP gave a substantial contribution to collect data, critical writing and revising the intellectual content. All Authors approved the final version.

Abstract

Introduction. The recently published ACR/EULAR classification criteria score (3 points or more) both clinical and laboratory criteria to define the presence of Antiphospholipid Syndrome (APS). The clinical criteria have been better defined while laboratory criteria remain the same [lupus anticoagulant (LA), anticardiolipin (aCL) and anti ß2-Glycoprotein I (aß2GPI) antibodies] but with different impact (points) on the classification of patients. APS is excluded if more than 3 years separate positive test for antiphospholipid antibodies (aPL) and clinical manifestation. ***Methods.*** The present study evaluates how many patients would be excluded by the new criteria among those enrolled as APS in the START 2 antiphospholipid registry. The analysis includes 380 patients (274 APS and 106 carriers). ***Results.*** Of 274 patients classified as APS, 118 (43%) did not match the new ACR/EULAR criteria for various reasons. First, the determination of aCL and aß2GPI antibodies was performed by automated instrumentations not allowed in the new criteria. Second, laboratory test score was less than 3 and this was due to an isolated IgM aCL or IgM aß2GPI in most cases and to isolated LA unconfirmed after 12 weeks in few cases. Third, 2 patients had a positive laboratory tests more than 3 years after the clinical event.

Of the 106 carriers, 62% had aCL and aß2GPI determined by ELISA thus meeting the ACL/EULAR laboratory criteria but were negative for clinical criteria.

Discussion This study shows that many patients classified as APS in the START 2 registry do not match the classification using the new ACR/EULAR criteria.

Key words: Classification, criteria, antiphospholipid syndrome, antiphospholipid antibodies

Introduction

The recently published ACR/EULAR classification criteria of Antiphospholipid Syndrome (APS) are the result of a rigorous four-step process (1). In phase I, 54 experts in the field generated candidate criteria. In phase II and III, there was a reduction of candidate criteria based on systematic reviews, meta-analyses, real world cases and expert consensus. Lastly, in phase IV data were validated using an independent adjudicators' consensus. As the previous revised 2006 Sapporo classification criteria (Sydney criteria)(2), the new criteria considered APS patients those with at least one clinical and one laboratory criterion (lupus anticoagulant-LA, IgG or IgM anticardiolipin-aCL and IgG or IgM anti ß2Glicoprotein I –aß2GPI). At variance with revised Sapporo criteria, the new criteria consider APS if less than 3 years (unlike the previous ~~revised Sapporo~~ criteria that considered 5 years) separate the antiphospholipid antibodies (aPL) positive test and the clinical manifestation. Clinical and laboratory criteria each receive a score that should be of 3 or more to classify a patient as having APS. The classification criteria are intended for the inclusion of a homogeneous population of participants in studies and trials although they have also been erroneously used as diagnostic criteria (3). The aim of this study was to ascertain how many patients would be excluded by the new criteria among those entered as APS in the START 2 antiphospholipid registry.

Methods

The Survey on AnTicoAgulated Patients- RegisTry (START) aPL registry is a prospective registry promoted by Arianna Foundation on Anticoagulation including long-term data collected by Centers for the diagnosis of thrombosis and surveillance of antithrombotic therapies(4). Subjects were recruited when testing positive for one or more criteria aPL (aCL, aß2GPI antibodies IgG/IgM), lupus anticoagulant (LA) and confirmed again after 12 weeks. The classification of patients was done according to Sidney clinical and laboratory criteria except for the immunological tests which were performed in some centers by automated systems, in most cases by chemiluminescence

immunoassay and Acustar instrumentation (Werfen group, Milan, Italy). According to the Sidney criteria, aCL and abeta2GPI were considered positive for enrollment in the registry if their value was above the cut-off calculated using the 99th percentile of that obtained in control subjects.

For LA determination, centers followed the recommendations that are summarized in a guidance document of the ISTH-SSC aPL subcommittee(5) and in a separate ISTH SSC guideline on LA testing in anticoagulated patients (6). However, we discouraged the participating Centers to bridge oral anticoagulants with LMWH for LA detection since even a short discontinuation of oral anticoagulation in high-risk cases might cause a catastrophic APS. Positivity of LAC was always confirmed after 12 weeks.

Demographic and clinical data (thrombosis, pregnancy complications, non-criteria clinical manifestations, systemic autoimmune disease, cardiovascular risk factors and treatment) were recorded in a web-based case report form. All patients enrolled in the study read and signed the informed consent. aPL profiles were classified as a) more than one aPL test positive and b) only one aPL positive test)(2).

Results

As of December 2023, the START2 Antiphospholipid registry has enrolled 380 patients. Of these, 274 were classified as APS, while 106 patients repeatedly positive for aPL did not have the clinical features of the syndrome (carriers).

Characteristics of APS and carriers are reported in Table 1.

Table 1. APL positive patients in the START2 antiphospholipid registry

	APS N=274	Carriers N=106
Age (yrs)	55±16	53±15
Female	172 (63)	75 (71)
Clinical criteria - VTE -Arterial Thrombosis -Obstetric APS	 149 (54) 88 (32) 37 (14)	 - - -
Laboratory criteria -More than one positive test -Just positive LA -Just positive aCL -Just positive antiß2GPI	 189 (69) 55 (20) 21 (8) 9 (3)	 76 (72) 27 (25) 2 (2) 1 (1)
Associated autoimmune diseases	88 (32)	37 (35)

± denotes mean and standard deviation; ( ) denotes percentage; APS: antiphospholipid syndrome; VTE: venous thromboembolism.

As shown in Figure 1, of the 274 APS patients who entered the registry, 118 (43%) did not match the ACR/EULAR classification criteria. Figure 2 shows reasons why patients reported as APS in START2 registry did not match the classification using the new ACR/EULAR criteria. In 49 patients (41%), aCL and aß2GPI were measured using automated instrumentations, a method not covered in the new criteria that indicate ELISA as the only recommended method. In 47 (40%), laboratory criteria score was less than 3 (in most cases just positive IgM aCL or IgM aß2GPI and in few cases isolated LA unconfirmed/doubtful after 12 weeks. In 12 cases (11%) the score for clinical domains was less than 3 (all cases had three or more unexplained consecutive spontaneous pregnancy losses before the

10th week of gestation). Eight patients (7%) had thromboembolic events in the presence of high risk VTE (n=6) and ATE (n=2) profiles. Of the 6 VTE events, 4 occurred during pregnancy/postpartum and 2 occurred in women while taking estrogen-containing oral contraceptives and associated systemic active autoimmune disease. Of the two patients with ATE, 1 had hyperlipidemia and the other suffered of arterial hypertension. Two patients had positive laboratory tests after more than 3 years from the clinical event.

Of the 106 carriers, 66 (62%) had aCL and aß2GPI positivity as determined by ELISA and all of them satisfied the ACL/EULAR laboratory criteria. However, no ACR/EULAR clinical criteria were present. Eight patients had thrombocytopenia (score =2 points) but no other clinical criteria.

Figure 1. Antiphospholipid Syndrome (APS) patient in the registry and their matching with American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) criteria.

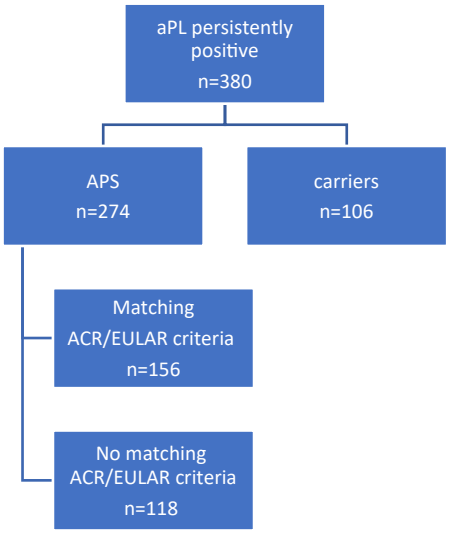
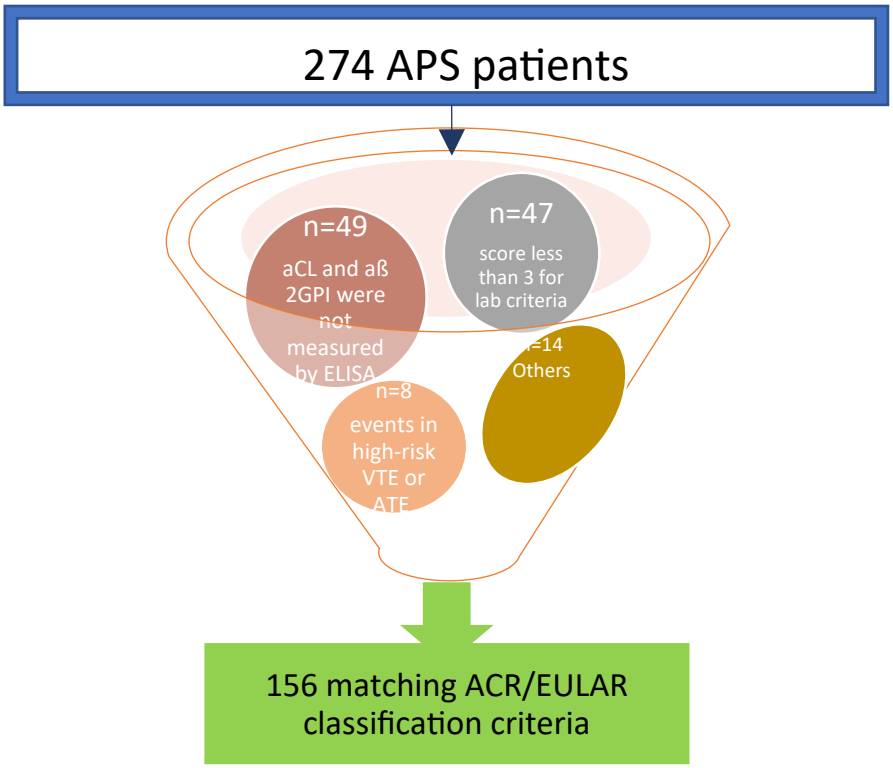


Figure 2. Effect of new classification criteria on 274 APS patients entered into START 2 Registry. aCL= anti-cardiolipin antibodies; aß2GPI= antibodies directed towards ß2-glycoprotein I; ELISA=

Enzyme Linked Immunosorbent Assay; VTE= Venous Thromboembolism; ATE= Arterial Thromboembolism.



Discussion

This study shows that many patients considered as having APS in START 2 Registry did not match classification using the new ACR/EULAR criteria. In 41% of cases, patients do not comply with new laboratory criteria since the immunological tests (aCL and aß2GPI) were performed using chemiluminescence assay (CLIA) or fluorescence enzyme immunoassay (FEIA) or other automated assays instead of the recommended enzyme-linked immunosorbent assay (ELISA). The extensive use of automated tests is justified since ELISA is affected by high variability (7) while non-ELISA assays are less time consuming and reduce intra- and inter-laboratory variation leading to higher reproducibility (8). ACL IgG/IgM results above 40 and 80 GPL/MPL units are considered as moderate and high levels (1, 9), but a correspondent level in non-ELISA assays is currently not available. Efforts to harmonize the interpretation of aCL and aß2GPI levels across various methods have been recently published by the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies (10). As the use of automated tools appears to be growing and irreversible, the entry of patients into clinical trials under the new classification criteria is heavily compromised. In 40% of cases, patients of START2 registry diagnosed as APS ~~based on revised Sapporo criteria~~ did not match the new ACL/EULAR classification because of lack of laboratory criteria. One point only is given for the presence of isolated IgM aCL or isolated IgM aß2GPI excluding these patients from new classification criteria. A long-lasting debate in the literature is present on this issue (11-14). Isolated LA not confirmed thereafter is given one point and is excluded. Indeed, isolated LA could be transient, falsely positive and not associated with thromboembolic events (15-17). Previous definition of obstetric APS included non-evidence-based definition of pregnancy morbidity resulting in the inclusion of a heterogeneous group of “aPL-positive” patients with different risk profiles for research (18). The exclusion of women with three or more consecutive spontaneous abortions before the 10th week of gestation determined the exclusion of 11 obstetric APS patients from our registry. The concept of thromboembolic events associated with aPL in the presence of important risk factors for

both arterial and venous thrombosis is appreciable as the presence of aPL might be irrelevant as the cause of thrombosis. Indeed, some patients in our registry fell in this category and did not match the classification criteria. Finally, the restriction to 3 years (instead of 5) between aPL determination and clinical events excluded 2 more patients from the new classification criteria. Most of the decisions taken by experts are shareable while other might generate further debate. A better definition of clinical events associated with APS is certainly useful while the definition and interpretation of laboratory data need further studies. In any case, the effort made may lead to more uniformity in including patients in clinical trials allowing a better generalization of results.

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