NAME OF PROJECT:

„Successive follow up of antiphospholipid antibodies fluctuations in patients with clinical Sydney criteria for APS: Long Term impact for diagnosis and outcome“

SKYLARK STUDY

Subcommittee
Lupus Anticoagulant/Antiphospholipid Antibodies of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis

Person responsible (Chair / Principal Investigators): Pr K Devreese/ Dr C Belizna, Pr H Cohen, Dr H Kelchtermans, Pr K Devreese

Description Abstract

The application is included in the broad remit of ISTH/SSC supported research and is a proposal for an international prospective antiphospholipid antibodies (aPL) titers follow-up.

Although the definition of antiphospholipid syndrome (APS) according to Sydney criteria resides in the positivity of aPL confirmed three months later (1), the time of the repeat aPL determination has been arbitrarily chosen. Particularly as tests exploring the presence of aPL are not standardized, reproducing the same results after three months renders test results more reliable. However, this approach delays the classification of patients, and may influence the choice and duration of antithrombotic treatment.

Moreover, different approaches for the initial detection of aPL vary depending on the clinical event (stroke, myocardial infarction, venous thromboembolism (VTE), obstetrical manifestations). This approach has several explanations, as some authors have suggested that there is a fall in aPL positivity at the time of thrombo-occlusive episodes (2); and others that, as the activation of endothelial cells (ECs) by aPL is an important mechanism that may precede thrombus formation in patients with APS (3), aPL detection at the time of diagnosis could represent only a marker of an endothelial lesion.

Therefore, in routine practice, although aPL are generally performed by the neurologists on stroke units in patients with cryptogenic stroke at diagnosis, most clinicians do not perform aPL testing when a clinical event such as venous thromboembolism (VTE) is diagnosed, and, if testing for aPL, do so only at the end of a course of anticoagulant treatment. Patients with VTE could also have aPL testing when they have specialist internist/vascular/haematological review; and obstetric morbidity patients have aPL testing when they get seen in clinic, subsequent to the obstetric event. Myocardial infarction patients do not
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routinely have aPL testing unless they get referred for specialist review, e.g. young, no evidence of atheroma.

However, deferral of aPL testing could be a source of underdiagnosis of APS, as aPL titers and lupus anticoagulant (LA) positivity could fluctuate over time (4-12), and with various treatment regimens (4, 13). Fluctuations in the titers of anticardiolipin antibodies (aCL) have been reported in systemic lupus erythematosus (SLE) patients (4-6). Moreover, under/delayed diagnosis of APS could also have major impact on the choice of the treatment. Vitamin K antagonists (VKAs) are the conventional anticoagulants for VTE patients with APS and while rivaroxaban has the potential to be an effective and safe alternative to warfarin for APS patients with a single VTE event requiring standard-intensity anticoagulation, further studies, particularly to provide better long-term efficacy and safety data, are needed (14, 15). VKAs, rather than DOACs are the preferred treatment in high risk APS patients, especially those who are triple positive or who have arterial or microvascular thrombosis (16, 17).

Data on changes in aPL titers during pregnancy and their influence on pregnancy outcomes are controversial (7-12). Few studies have reported serial aPL determinations through pregnancy (7-12). Some authors found no relationship between aPL fluctuations with adverse pregnancy outcomes (7-9), while others reported favorable pregnancy outcomes associated with falling titers of aPL (10-12).

The value and optimal timing of repeated testing for aPL requires further definition.

One study further reported that confirmation or repeat testing in all APS patients may not be of added value in triple aPL positive patients (18). Moreover, our preliminary observations in a French cohort suggest that aPL titers fluctuate during follow-up in obstetrical and thrombotic APS, but also that the optimal timing for aPL detection remains a major clinical need, as in some cases, the initial positive aPL could be confirmed at 1, 2, 6 months and more than 6 months, although they were not detected at 3 months (unpublished data).

Definition of the optimal timing of the first determination for aPL status and of LA positivity confirmation, to establish persistence of aPL, the correlations of aPL titer fluctuations and LA positivity with clinico-biological features and various treatments in APS would be expected to have a major impact in clinical practice and to provide the answer to an unmet clinical need.

Based on the above considerations, we propose an international multicentre prospective study of follow up for 3 years of aPL titers and LA positivity in patients with clinical criteria of APS according to Sydney criteria.

The primary aim of this study is to evaluate the optimal time/time range to test for aPL: during the acute event or at 1, 2, 3 or 6 months after a clinical event (VTE, stroke, myocardial infarction, pregnancy morbidity such as fetal loss or recurrent miscarriages).

Secondary aims will be:
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- To evaluate the optimal time to do the aPL tests for positivity confirmation: at 1, 2, 3 or 6 months after the first positive result
- To evaluate the eventual clinical benefit of serial repetitive aPL tests
  - every 3 months during pregnancy in patients with previous pregnancy morbidity such as fetal loss, recurrent miscarriages (during the pregnancy at Month M3, M6, and at the end of pregnancy, the latest at M9); and subsequently at M12, M18, M24
  - and every 6 months in patients with various clinical events (VTE, stroke, myocardial infarction, previous obstetrical event) during the follow-up period of the trial (M6, M12, M18, M24).
- To evaluate if the confirmatory or repeat testing may or may not be of added value in triple aPL positive patients
- To characterize the clinical and biological features of patients associated with the aPL titers fluctuations defined as variations of more than 20% compared to former values
- To evaluate whether the fluctuations of aPL titers are correlated with treatments, outcome, new clinical events, complications and survival.

Design and methodology (Data expected to collect, sample size and statistical analysis):

An international multicentre prospective study will be undertaken over 3 years (one year of recruitment and two years of follow-up). The study population will comprise patients with clinical criteria of APS with either thrombotic or obstetrical manifestations (incident cases), positive for a first determination of aPL. Consecutive patients referred to recruiting centers who will be initially positive in one or more tests exploring the presence of aPL, lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), anti-β2-glycoprotein 1 (antiβ2-GPI) antibodies will be serially tested. The first aPL determination will be performed at diagnosis of the clinical event which is Time 0.

The participating centers will be the expert centers led by the LA/aPL SSC Co-chairs who have agreed to participate in this project. Other centers would be approached via the LA/aPL SSC website from the outset to maximize recruitment. Several young investigators from each participating centre are involved in this study.

Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

The inclusion criteria will be:

- Patients positive at initial diagnosis for at least one aPL type associated with arterial, or venous, or obstetrical event according to Sydney clinical criteria (1)
- Age > 18 years
- Patients are volunteers, informed and signed the consent form for participation in the register after receiving the information letter.

Criteria for exclusion
Considering the number of centres that have agreed to participate to date and the period of recruitment, we plan to collect data from 200 patients.

Specialists from multidisciplinary teams (haematologists, specialists in haemostasis and thrombosis, internists, rheumatologists, biologists, immunologists, neurologists, cardiologists) will belong to international centres from several countries from all co-chairpersons of the SSC who agreed to participate in this study.

In all participating centers the Principal Investigator will inform the other specialists about the trial, as it is not routine to test aPL at all the time points specified.

Recruitment cannot be performed at a first visit of a patient showing clinical features related to APS, but after receiving the aPL results. The patient shall be recalled for a second visit, after one month. Time 0 will correspond to the aPL first determination.

Therefore, the recruitment will be mainly performed:

- via the Emergency Unit and Cardiology unit in each center for VTE and MI in young patients (<50years of age), without obvious conventional cardiovascular risk factors
- via the Neurology stroke unit in young patients with cryptogenic stroke
- via the Haematology department
- via the Obstetrics/Gynecology unit.

All the other specialists willing to participate could also include patients with typical APS clinical manifestations.

The investigator will check for eligibility inclusion criteria and give information about the study (orally and Patient Information Sheet) at visit after one month (M1). If agreement, patients will sign an informed consent. After informing the patient and obtaining his/her signature on the informed consent form, the investigator will fill in a computerized inclusion form, available 24/7 over the Internet via the e-CRF (secure access previously granted to each pre-declared investigating centre).

Blood samples will be collected for aPL. These tests are not included in the routine follow-up of the patients. Blood tests will be scheduled for patients with thrombosis and/or previous obstetrical events at M1, M2, M3, M6, M12, M18, M24 and clinical visits will be performed at M1 and every 6 months.

One additional aPL determination will be performed in pregnant women, at the delivery, the latest at M9.
Obstetrical patients will be evaluated monthly by an obstetrician and each trimester by a specialist in APS through three months postpartum with physician examination, obstetric ultrasounds and laboratory testing according to current practice in managing these patients.

Data will be collected with respect to aPL positivity and titers, patient's age, sex, type of clinical manifestations, type of treatment, response to treatment, number of recurrent episodes of thrombosis or pregnancy morbidity, number of unscheduled hospitalizations, number of side effects (bleeding) at inclusion, and during follow-up.

All data will be recorded at 7 time points: at 1, 2, 3, 6 months and every 6 months by each investigator (M1, M2, M3, M6, M12, M18, M24) in all patients, and additionally, at the delivery in pregnant patients.

All investigators agreeing to participate will add these tests preferentially during a routine follow-up blood test. The study tests will require for each time point one additional 8 ml citrated blood sample. Blood samples will be immediately processed (double centrifugation and frozen at -80°C in three aliquots until testing) and shipped on dry ice to the core lab.

To avoid variability of tests between the commercially available aCL and antiβ2GPI assays: each test result for aPL above the cutoff value calculated as higher than the 99th percentile, would be considered as positive. We will also look at the 95th percentile as low positive aCL and antiβ2GPI results may be clinically relevant in obstetric APS.

Moreover, the centralization of all aPL tests send to the Core Laboratory center, Coagulation Laboratory-Ghent University Hospital, Belgium, will enable homogeneity of the results and thus avoidance of the high variability of test results between different centres. The transport fees will be covered by the grant.

LAC, aCL and antiβ2GPI will be performed according to the SSC recommendations (19, 20) and British BCSH/BSH guidelines (21).

As the trial involves interventional biomedical research, it shall be declared to the competent authorities in each country and shall be subject to IRB approval.

The statistical analysis will be undertaken using the software Excel version 2010 of Microsoft (Redmond Washington the United States) and of the software IBM SPSS statistics version 23 (Chicago Illinois the United States).

Median titers and median changes in titers during follow-up will be compared to those at screening using the Wilcoxon-Paired test. Variation in aPL positivity of more than 20% compared with the former value during follow-up will be analyzed using the Mac Nemar test for paired-samples, and univariate analysis of factors associated with new clinical events will be performed with the Chi-square, Fisher’s exact tests for categorical variables and Mann-Whitney test for continuous variables. Statistical testing will be done at the two-tailed α level of 0.05.

The Fisher’s exact test will be used to evaluate bivariate associations between categorical variables. Multivariable analyses will be performed by fitting a Poisson regression model with a robust error
variance to estimate the relative risk for an exposure variable adjusted for the effects of other covariates and corresponding 95% confidence intervals (22).

Variables which will be predictive of new events in bivariate analyses (p < 0.20) as well as those deemed to be associated with new events a priori based on clinical factors, will be considered for inclusion in the model. The final model will be determined using a backward selection approach and included only those covariates which remained significant at the p< 0.05 level. An internal validation of the predictive model will be also performed using a 5-fold cross-validation procedure. The model will be re-fit at each step of the validation using the training data, and evaluated on the corresponding test set. Results of the validation procedure will be reported as the sensitivity, specificity, and accuracy of the model averaged over all test sets.

For the comparative analysis of the groups, the Student t test will be used for the quantitative variables, having verified beforehand the equality of the variances for the studied parameters, and the test of Chi-square for the nominal qualitative variables.

Collected variables will be described globally and per group. Qualitative variables will be expressed in population size and percentage. Quantitative variables are expressed in terms of mean ± standard deviation with 95% confidence interval, along with the 5th and 95th percentiles. These are, however, expressed in terms of median, minimum, maximum and 5th and 95th percentiles when normality is rejected. The Kolmogorov- Smirnov test will be used to check parameter normality and the Levene test will be used to determine equality of variances. An alpha risk of error of 5% with a significance for p of 0.05 will be admitted. The analysis of one of the outcome measures shall consist in comparing the proportions of subjects with new clinical events, and with different prognostic profiles in the groups of patients with different clinical profiles. This comparison will be performed by means of a Chi-square test, or a Fisher exact test if the Chi-square application conditions are not met. If a difference between groups is demonstrated for at least one of the potential confounding variables, the main outcome measure shall be analysed by means of multivariate logistic regression to consider the adjustment factors. Furthermore, the number of events in each group will be compared, with a Poisson regression model and a negative binomial regression model. The search for correlation will use the calculation of the coefficient rho of Spearman in case of the low size of groups, the not parametric character and the not normal distribution of the data.

We will perform statistical comparative analysis based on lupus anticoagulant positivity, on type of antiphospholipid antibodies (IgG and IgM aCL, IgG and IgM antiβ2GPI antibodies) and values.

A survival analysis related to the risk of clinical events and to the risk of relapse associated with aPL during follow-up, will be performed and estimate by calculating the hazard ratio (Cox model). Descriptions for time to adverse events including new morbidity events using Kaplan-Meier methods for evaluation will be used to support interpretation of results.

One major issue consists of variability in results between different laboratories, and one of the strategies to overcome this issue will be that the results for aCL and antiβ2GPI antibodies will be expressed as positivity above the 99th centile. Moreover, the strength of our study is based on the centralization of all
the tests sent to the Core Laboratory center, Coagulation Laboratory-Ghent University Hospital, Belgium, which will ensure the homogeneity of the results for this test in order to avoid high variability of tests.

The recruitment target is attainable as we expect that 600 patients with various clinical profiles from the recruiting centers will be tested. Some recruiting centers have their own ongoing biological collections. Patients will be recruited after the initial test is done: only aPL positive patients will be enrolled (200 patients are expected to be included and to complete the whole follow-up).

Moreover, the participation of expert centers, leaders in APS and with a high potential for recruitment should assure the feasibility of this study. Although the feasibility of collecting serial data from an important number of patients will be challenging, we are optimistic as our consortium succeeded in performing several studies on large cohorts, last in date being represented by another international retrospective and prospective trial, HIBISCUS, which includes at now more than 700 patients with primary APS from 53 centers in 16 countries (23, 24).

**Expected timeline: Project stage/set up**

- Finalization and set-up of the project: March 2019
- Launch of the project: October 2019
  - Duration: 36 months
    - Inclusion: 12 months
    - Follow-up: 24 months
- Completion/statistical analysis: October 2022
- Reporting: research results and project milestones will be reported at the Subcommittee’s annual SSC meeting and annual progress report made to the Executive Committee starting July 2020

**Expected outcomes**

The results will be submitted as an ISTH-SSC publication, both SSC Communication and original research on behalf of the SSC. Depending on the outcome, a Recommendation of the SSC could also be proposed.

All publications resulting from the research will be published in an ISTH journal.

Research results and project milestones should be reported at the Subcommittee’s annual SSC meeting and an annual progress report made to the Executive Committee.

The results of this longitudinal study will have potential clinical consequences on the diagnosis, screening and therapeutic regimens in APS and particularly in the high risk identified subgroups of patients.

We strongly believe that APS is an underdiagnosed disease and, as the early and reliable diagnosis is essential to help determine the prognosis and guide appropriate treatment of these patients, most of them young (mean age 20-40 years), we anticipate that this proposal would provide an answer to an unmet medical need.
We anticipate that this project will lead to additional future work by the Subcommittee. This project will enable us to create a homogenous biological and clinical well characterized APS cohort. The project and the constitution of a biological international cohort will allow new projects focused on additional data with respect to anti domain1 GP1 detection in these patients, the role of IgA anticardiolipin and antiB2GP1 antibodies. It will also allow the better characterization of this population in further studies and, also, additional studies on the predictive value of different biological and clinical data for the outcome and response to treatments.

Description of project set-up and management, needed infrastructure and resources:

The project will be led by the Lupus anticoagulant SSC subcommittee. Several young investigators from each participating centre are involved in this study. A computerized register with an inclusion form, available 24/7 over the Internet via the e-CRF (secure access previously granted to each pre-declared investigating centre) will be created. ISTH REDCap installation will be employed as technical support for the project. All data will be recorded at 7 time points: at 1, 2, 3, 6 months and every 6 months by each investigator (M1, M2, M3, M6, M12, M18, M24) in all patients, and, additionally, at the date of delivery in pregnant patients.

The major hurdle will be funding for aPL tests, given the number of tests to be done on each patient, the frequency of which is not standard in routine practice. Funding will be needed for serial aPL tests, reagents, samples transportation for aPL testing in the Core Laboratory Center, a part time laboratory technician, a part time data manager, and a part time methodologist/ biostatistician. The grant is intended to cover only a part of the costs and we have therefore contacted the manufacturers to provide the reagents for free of charge (which has been achieved for other multicenter aPL projects that the Investigators are undertaking). Complementary sources of funding are considered: Lupus patients association (AFL+, France and others), E-Rare grant, Lupus foundation of America,…

The dissemination of the project results will be through communications in congress and international conferences and via different publications.

References:


