

## Commentry

# Alternative for Paraclinical Diagnosis in Suspected Antiphospholipid Antibody Syndrome in Women with Arrested Pregnancies

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## Abstract

**Introduction:** Antiphospholipid antibodies are a heterogeneous group of circulating antibodies that recognize phospholipids and phospholipid-binding proteins as antigen. Anti-phospholipid antibodies have been found in 6% of women with obstetrical pathologies. In Madagascar, no studies have been conducted regarding the prevalence of antiphospholipid antibodies in terminated pregnancies. The objective of our study is to find an alternative for the paraclinical diagnosis of antiphospholipid antibodies in women with unexplained recurrent pregnancies at the University Hospital of Gynecology and Obstetrics Befelatanana (CHUGOB).

**Material and Methods:** The study was a prospective descriptive study on the search for lupus anticoagulant in all patients with an arrested pregnancy who were admitted to the gynecology department of CHUGOB during the month of March 18, 2019 to October 09, 2019. Epidemiological parameters were studied such as age, gestational age, parity, number of abortions but also medical history and biological parameters prolongation of the aPTT and elevated RPR.

**Results:** During the study period, During our study period we included 100 patients, The median gestational age of our study population was 27 days with a minimum of 10 days and a maximum of 40 days with a predominance of spontaneous abortions (55%) over in-utero fetal deaths (45%). In the biological analyses, 3% of the patients had an APTT ratio greater than 1.2. After correction, one case had a Rosner index of 29.1. Three cases of RPR positivity with a low titre without TPHA positivity were found. One patient with disseminated lupus erythematosus had an uncorrected prolonged APTT and a positive RPR with a low titre.

**Conclusion:** the prolonged aPTT, the use of the false positive syphilis serological test for the detection of aCL can be an inexpensive alternative, but does not allow the presence of aCL to be affirmed with certainty

**Keywords:** recurrent pregnancies, Antiphospholipid antibodies, activated cephalin time, Madagascar

## 1. Introduction

Antiphospholipid antibodies are a heterogeneous group of circulating antibodies that recognize phospholipids and phospholipid-binding proteins as antigen [1]. The persistent presence of anti-phospholipid antibodies associated with thrombotic clinical manifestations and/or obstetrical complications characterizes the Anti-Phospholipid Syndrome or APS [2]. The incidence of APS in the general population remains unknown. The prevalence is 40 to 50 cases per 100,000 in Western countries [3]. In the United States, anti-phospholipid antibodies have been found in 6% of women with obstetrical pathologies (early or severe preeclampsia, severe placental insufficiency, *in utero* fetal death, intrauterine growth retardation and recurrent abortion) [3]. In Madagascar, according to a 2010 estimate by the Ministry of Public Health, there are approximately 75,000 cases of abortion per year. However, no studies have been conducted regarding the prevalence of antiphospholipid antibodies in terminated pregnancies.

The detection of anti-phospholipid antibodies is done by phospholipid-dependent coagulation tests and by immunological techniques. The main antiphospholipid antibodies detected in this way are: lupus anticoagulant (LA), anticardiolipin (aCL) and anti- $\beta$ 2-glycoprotein I ( $\alpha\beta$ 2GPI) antibodies [1]. These tests are expensive and not accessible to the majority of the population. The objective of our study is to find an alternative for the paraclinical diagnosis of antiphospholipid antibodies in women with unexplained recurrent pregnancies at the University Hospital of Gynecology and Obstetrics Befelatanana (UHGOB).

## 2. Method

This is a prospective descriptive study on the search for lupus anticoagulant in all patients with miscarriage who were admitted to the gynecology department of UHGOB during the month of March 18, 2019 to October 09, 2019.

We included in the study patients with early spontaneous abortion, late spontaneous abortion and fetal death in utero during the study period. We excluded women with an abortion inferior to 10WA. But also all patients with pathologies and/or factors inducing a prolongation of the aPTT (*eg.* drug intake such as AVK, heparin therapy, neuroleptics but also documented coagulation pathologies) but also documented causes of elevated RPR results, especially syphilis. Epidemiological parameters were studied such as age, gestational age, parity, number of abortions but also medical history (hypertension, diabetes, smoking, history of oral contraceptive use and systemic lupus erythematosus). To identify the presence of anticoagulant lupus, we proposed to look for the presence of anti-cardiolipin antibodies by the Rapid Plasma Antigen (RPR) and by the activated cephalin time (APTT) uncorrected after addition of control plasma.

Patients included in the study were collected on a dry, citrated 5-ml tube where we looked for:

- Rapid Plasma Reagin RPR (RAPIDS LABS® UK), according to the manufacturer's recommendations were considered positive the presence of agglutination visible to the naked eye with a titer  $\geq 8$  IU/mL and for the titration of positive samples, the same method was applied using double serial dilutions of the samples in saline at 9 g/Dl.
- Treponema Pallidum Agglutination Assay (RAPIDS LABS® UK) to rule out syphilitic infection.
- Hemostat aPTT-EL (RAPIDS LABS® UK) on semi-automatic machine SStart® 4 from Stago Diagnostica. The search for lupus anticoagulant is suspected if the aPTT is not corrected after addition of control plasma.

We suspected anticardiolipin antibodies and lupus anticoagulant in the presence of positive RPR and negative TPHA associated with an uncorrected prolonged aPTT. The data were collected on Microsoft office Excel 2013 and the statistical analysis of the data was performed with the R software, the threshold value of  $p$  is 5%. Chi-square test was used to compare the observed percentages. Student's t-test was used to compare means. Measures were taken to ensure strict confidentiality in the preparation of the records. The study was conducted with the patient's signed consent.

## 3. Results

During our study period we included 100 patients, with an average age of 28.02 years with a minimum of 17 years and a maximum of 48 years. The age range 20-29 years was predominant at 55%. The median gestational age of our study population was 27 days with a minimum of 10 days and a maximum of 40 days with a predominance of spontaneous abortions (55%) over in-utero fetal deaths (45%). A predominance of pauci gestations (39%) and pauciparous (42%) were observed (Table 1). Population with no particular medical history were predominant with 85% of cases. Twelve patients were on oral contraception. The presence of 4% of cases with repeated spontaneous abortion was observed. In the biological analyses, 3% of the patients had an APTT ratio greater than 1.2. After correction, one case had a Rosner index of 29.1. Three cases of RPR positivity with a low titer without TPHA

positivity were found. One patient with disseminated lupus erythematosus had an uncorrected prolonged APTT and a positive RPR with a low titre.

**Table 1:** Number of abortions in our patients

abortions	Number	Percentage(%)
0	40	40
1-2	56	56
≥3	4	4
Total	100	100

#### 4. Discussion

In our study, the prolonged APTT with a ratio greater than 1.2 was used as a screening test. The absence of the inhibitory effect when mixing equal volumes of normal plasma and pathological plasma to be corrected would point to anticoagulant lupus with the calculation of the Rosner index which must be greater than or equal to 15. The results of our study had shown that 3% of cases have prolongation of the APTT, 1% uncorrected and suspected to be positive for lupus anticoagulant. These data are similar to that of previous study conducted in Japan by Yamada among 1157 women with an active pregnancy, collected at 8-14 WA revealed that the frequency of positivity of lupus anticoagulant to 0.8% [4].

Lupus anticoagulant, like all antiphospholipid antibodies, predominates in the Caucasian population, which explains the high positivity in some countries, as in the study of Zachary et al, in the United States, on a larger population than ours, including recurrent spontaneous abortions less than 10 weeks' gestation, fetal loss beyond 10 weeks' gestation, a history of systemic lupus erythematosus, severe pre-eclampsia and placental insufficiency less than 34 weeks' gestation, showed that out of 335 patients, 8 were positive for lupus anticoagulant, i.e., a positivity of 2.38% [5] In Canada, a study conducted by Clark et al. among patients with recurrent spontaneous abortion showed that 62 (2.7%) of 2257 cases were positive for lupus anticoagulantum [6].

The positivity of LA varies considerably according to the obstetrical complications where they are sought, indeed frequent in severe or early pre-eclampsia than in miscarriage. This would explain the increased positivity of LA testing in some studies. The study by Ankur et al recruiting patients at high risk of APS (recurrent spontaneous abortion, women with HELLP syndrome, Eclampsia, severe pre-eclampsia) showed that out of 526 patients, 40 were positive for lupus anticoagulant, i.e. 7.6% [7]. In Nigeria, a study by Ibrahim et al, concerning patients with spontaneous abortion before 28 weeks of amenorrhea revealed that out of 100 cases, 8 presented a prolongation of the APTT, 4 of which were uncorrected by the addition of control plasma (i.e. 4%), these 4 cases were confirmed by the time of Russel's viper venom [8].

Our result of 1% positivity for LA may be underestimated because the sensitivity of the TCA test alone is 50-70% of cases, not available diluted Russell's viper venom time according to the ISTH recommendation of 2009. However, according to a study in Singapore, the main cause of APTT prolongation was related to the presence of lupus anticoagulant in 53.1% of cases [9]. However, the search for uncorrected APTT prolongation alone can therefore be used as an inexpensive diagnostic tool to suspect the presence of LA in the face of strong clinical suspicion, its negativity does not however completely rule out the presence of LA. Anti-cardiolipin antibodies were first discovered as a result of a false positive test for syphilis. A study in the USA by Murphy et al. using RPR and TPHA for the detection of rheumatic diseases revealed a specificity of 91.9% [10]. A study by Henriksen et al, in Sweden, recruiting patients with spontaneous abortion and a false positive RPR showed the presence of anticardiolipin antibodies [11].

Other causes of false positive syphilis serology tests have not been eliminated in our study, these other causes are multiple and varied, a Chinese study by Fan et al on the characterization of the classical biological false positive syphilis serology test has indeed shown that a relationship with more than 60 different diseases has been established with these false positives but the most frequent were malignant tumors, systemic lupus erythematosus, urticaria, dermatitis and eczema [12]. The false positive syphilis serology test has a low sensitivity for the detection of aCL, in fact a study in the United States evaluated its sensitivity at 62.5% [10].

In conclusion, as for the prolonged aPTT, the use of the false positive syphilis serological test for the detection of aCL can be an inexpensive alternative, but does not allow the presence of aCL to be affirmed for sure. However, the case of our patient with lupus erythematosus with positivity of both tests reveals the effectiveness of these less expensive screening alternatives, to be confirmed later by more sensitive techniques.

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