# The prevalence of lupus anticoagulant in normal pregnancy and in women with recurrent fetal loss—recommendations for laboratory testing for lupus anticoagulant

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**BACKGROUND:** There is wide disagreement in the literature on the rate of detection of lupus anticoagulant (LA) in women with recurrent fetal loss (RFL). The aim of this study was to determine the prevalence of LA using four phospholipid-dependant coagulation tests in a large population of Saudi women.

**PATIENTS AND METHODS:** We determined the prevalence of LA in women with RFL (n=925), normal pregnancy (n=663), and in healthy blood donors (n=204), at the King Khalid University Hospital, Riyadh. The following coagulation tests were employed: the activated partial thromboplastin time (APTT), platelet neutralization procedure (PNP), kaolin clotting time (KCT) and the dilute Russel's viper venom test (dRVVT).

**RESULTS:** In RFL patients, positive APTT was 10.2%, APTT+PNP 3.6%, KCT 10.5%, and dRVVT 10.9%. In normal pregnancy, the corresponding figures were 12.8%, 3.1%, 10.8%, and 5.6%. Three positive tests occurred in 2.3% of RFL patients, including APTT+KCT 3.5%, APTT+dRVVT 3.9%, and KCT+dRVVT 4.1%. The corresponding figures for normal pregnancy were 1.6% for three positive tests, and 3.0%, 1.8%, 2.4%, respectively. The dRVVT was the only test that showed a rate of positive results almost double that seen in normal pregnancy.

**CONCLUSIONS:** If only one or even two screening tests were performed, a significant number of LA positive cases would have been missed. This could make a difference to treating physicians as to the possible etiology and management of RFL. It is therefore advisable to routinely use the three tests (APTT, KCT and dRRVT) when screening for LA.

**KEYWORDS:** Antiphospholipid antibodies, lupus anticoagulant, RFL, pregnancy

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Autoimmune mechanisms, including antiphospholipid antibodies (APAs) such as lupus anticoagulant (LA) and anticardiolipin antibodies (ACAs), are considered risk factors for recurrent fetal loss (RFL).[1] Antiphospholipid antibodies were originally described in patients with systemic lupus erythromatosis (SLE),[2] but were also detected in non-SLE disorders,[3] as well as in healthy individuals.[4] Lupus anticoagulant like ACAs are a heterogeneous group of immunoglobulins that bind to negatively charged phospholipids. Some earlier studies succeeded in establishing the association between APAs and low birth weight, intrauterine fetal death and adverse complications of pregnancy.[5-8] It is also being recognized that APAs are associated with four major clinical manifestations: arterial and venous thrombotic events, recurrent pregnancy loss and thrombocytopenia, and the so-called antiphospholipid syndrome.[9] Nonetheless, invoking the APA hypothesis and its thrombogenic role in the aetiology of RFL has given strong support to the use of anticoagulant/antiplatelets (heparin and aspirin) therapy in the successful management of women with recurrent pregnancy loss.[10-11]

In the laboratory, lupus anticoagulants act by prolonging the phospholipid dependent clotting tests.[5,12] However, there are wide variations in the use of guidelines set by the ISTH[13] in various published studies and the lack of conformation may account for the wide disparity in the documentation of the prevalence of the antibodies in RFL.

In the current study we have undertaken assays of LA in a large population of normal pregnant women as well as in patients with RFL, employing multiple coagulation tests, thereby maximizing the chances of detecting these heterogeneous antibodies, like no study has done before. It is our aim to test the sensitivity of employing one, two or the three currently widely employed coagulation tests (APTT, KCT and dRVVT), in characterizing women positive for LA. This will allow a more accurate assessment of the prevalence of lupus anticoagulant in our population.

This communication is part of a large project investigating the various etiological factors of RFL in Saudi Arabia.

## Patients and Methods

Nine hundred and twenty-five (925) women with recurrent fetal loss (RFL) were recruited consecutively from a special outpatient RFL Clinic, King Khalid University Hospital, Riyadh. These women were subjected to a detailed interview and the information was collected in a specially designed form. They underwent a general medical examination and extensive laboratory and radiological tests. Only those with no proven systemic or local gynecological cause of RFL were included. These patients gave a history of recurrent abortions, ranging from 3 to 15. Their ages ranged from 19 to 40 years (mean ±SD, 29.9 ± 6.5); the majority of the women (67.5%) were in their peak reproductive age group (25-34 years). The blood samples for this study were collected when these women were non-pregnant and under investigation as to the possible etiological role of lupus anticoagulant in their RFL.

Six hundred and sixty-three (663) women with a trouble-free pregnancy were recruited from the antenatal clinic at King Khalid University Hospital, Riyadh. Their ages ranged from 14 to 45 years, (mean ± SD, 28.8 ± 6.1). These women were further subdivided according to gestational age (gestational age was calculated from the date of the last menstrual period in conjunction with the findings of an ultrasound scan) into three subgroups as follows: first trimester (n=132), second trimester (n=293), third trimester (n=238). Healthy controls (n = 204) were blood donors of both sexes.

Venous blood samples were collected in sodium citrate (0.11M) to give a blood citrate ratio of 9:1. Samples were transported without delay to the coagulation laboratory. A 15-minute double centrifugation at 3000 rpm was done before separating plasma by double centrifugation as follows: centrifugation of citrated blood at 3000 rpm for 15 min; a plastic transfer pipette was then used to remove the supernatant platelet rich plasma, which was placed in a second plastic tube and recentrifuged under identical conditions. The resulting platelet poor plasma (PPP) was either tested immediately or stored in aliquots in the frozen state at -40°C for testing at a later date.

Assay techniques for detecting lupus anticoagulant followed the guidelines of the Scientific and Standardisation Committee of the International Society for Thrombosis and Haemostasis (ISTH), with respect to both pre-analytical and analytical variables.[13] The activated partial thromboplastin time (APTT)[14] was performed using a sensitive APTT reagent (APTT Manchester Comparative Reagents - UK) according to the manufacturer's instructions. The clotting time was recorded in duplicates using a coagulometer (Amelung Coagulometer-KC4A, Germany). The test is taken as positive if the patient's APTT is more than 2 SDs (3.5 sec) above the mean (48±7 seconds) of the normal range for healthy population, which is 55 (47+7= 55 seconds). Mixing with normal pool plasma was undertaken in samples showing >5-second prolongation of the APTT and those who did not show a correction were tested further with the platelet neutralization procedure (PNP) (see below).

The Kaolin Clotting Time (KCT) was performed according to the method of Extner et al.[15] An index of 10% or more is considered positive for the presence of LA. The Dilute Russel's Viper Venom Test (dRVVT) was performed according to the method of Thiagarajan et al.[16] The venom (Manchester Comparative Reagents , UK) was reconstituted using Tris Buffered Saline, pH 7.5. The result is taken as positive for lupus anticoagulant if the index of patient clotting time divided by control plasma clotting time is >1.1. The Platelet Neutralization Procedure (PNP)[14] was performed in conjunction with APTT. Shortening of the APTT by < 5 sec in the reaction mixture as compared to the saline control is considered confirmatory of positive for LA. The results of all tests were recorded in duplicates. These tests were done only once, when the patient presented the first time for investigation.

The Chi-square test was employed to determine the significance of differences in the prevalence of LA, based of the results of different laboratory tests. The prevalence of LA in normal pregnancy (Table 1) is shown for individual tests to facilitate the comparisons with other studies as well as those obtained in our RFL patients.

## Results

When comparing the prevalence of LA between women with normal pregnancy or blood donors and those with RFL, dRVVT is the only test that showed a markedly higher prevalence of LA among women with RFL (10.9%) than normal pregnancy (5.6%; $P<0.0279$), or healthy blood donors (1.2%; $P<0.001$). For RFL patients, the prevalence of LA was slightly higher for dRVVT (10.9%) when compared to the either the APTT (10.2%) or the KCT (10.5%), the values for any of the three tests being remarkably higher than in blood donors. For the combination of various screening tests, the prevalence of LA dropped markedly for both RFL patients as well as normal pregnancy, indicating that a patient who had a positive result for LA for one test will most probably have a negative result for the other two screening tests. Due to certain constraints, the PNP was employed only with the APTT and the results show that the prevalence of LA drops by approximately two thirds in both normal pregnant women and in patients with RFL, when the two tests are performed in those who were positive for APTT.

## Discussion

The prevalence of lupus anticoagulant in normal pregnancy[12,17-20] varies from 1.2% using the KCT test[20] to 0.27% us-

**Table 1.** Prevalence of lupus anticoagulants in normal pregnancy and in women with recurrent fetal loss.

| | Prevalence of lupus anticoagulants (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test | Normal pregnancy (1st trimester) n=132 | Normal pregnancy (2nd trimester) n=293 | Normal pregnancy (3rd trimester) n=238 | Normal pregnancy (total) n=663 | Recurrent fetal loss n=925 | Blood donors n=204 | Normal pregnancy vs recurrent fetal loss *P* value | Blood donors vs recurrent fetal loss *P* value |
| APTT | 10.6 | 13.4 | 12.6 | 12.8 | 10.2 | 3.0 | 0.001* | 0.0043* |
| APTT +PNP | 3.8 | 2.4 | 1.3 | 3.1 | 3.6 | 0.0 | – | – |
| KCT | 10.9 | 12.6 | 9.1 | 10.8 | 10.5 | 1.2 | 0.001* | 0.0002 |
| dRVVT | 6.8 | 4.8 | 5.2 | 5.6 | 10.9 | 1.2 | 0.0279* | 0.001* |
| 3 positive tests | 1.5 | 1.7 | 0 | 1.6 | 2.3 | 0.0 | 0.1333 | 0.0603 |
| APTT +KCT | 3.0 | 4.8 | 1.3 | 3.0 | 3.5 | 2.4 | 0.8006 | 0.6194 |
| APTT +dRVVT | 3.0 | 2.1 | 0.4 | 1.8 | 3.9 | 0.6 | 0.4839 | 0.0511 |
| KCT +dRVVT | 3.0 | 1.7 | 0 | 2.4 | 4.0 | 0.6 | 0.2196 | 0.0459* |

*Statistically significant at 5% level.

**Table 2.** The prevalence of lupus anticoagulant in RFL in earlier studies.

| Previous studies | APTT | KCT | DRVVT |
|---|---|---|---|
| Ria et al (17) (n= 300) | 1.0% | 1.0% | 6.7% |
| Parke et al (19) (n=81) | 4.9% | ND | ND |
| Maclean et al (12) (n= 243) | 1.2% | 1.2% | 5.67% |
| Infante-Rivard et al (23) n=331 | 5.1% (Either APPT or dRRVT +PNP) | | |
| The current study (n=925) | 10.2% | 10.5% | 10.9% |

ing the APTT.[17] These inhibitors are a heterogeneous group of antiphospholipid antibodies, and therefore we assume that when more screening tests are done (employing more phospholipid antigens) there is a greater chance of picking these antibodies than when employing a single test. It has already been shown that some weak to moderate LAs were detected with one but not another assay[21] and that each assay seems to detect a sub-population of LAs not readily detected by other assays.

In a recent comparative study employing two commercial assay techniques for the detection of LA, neither test alone detected more than 24 of 30 LAs but together the two assays detected 29 of 30.[22] It is recommended that at least two tests be performed to confirm the presence of LA.[13] This recommendation received strong emphasis in the earlier Guidelines for Testing for Lupus Anticoagulant, published by the Scientific Standardization Committee working under the auspices of the International Society for Thrombosis and Haemostasis (ISTH).[13] The subcommittee recognized that there is some variability in the diagnosis of LAs, a fact related to both the biologic heterogeneity and analytical variability inherent in the multiple assay systems currently in use. These methodological difficulties no doubt account for the wide variation in the results of the LA screening tests when more than one test is performed. This is also apparent in the wide variation in the prevalence of LA in normal pregnancy in this study (Table 1).

In our study, the prevalence rates for lupus anticoagulant in 925 patients with recurrent fetal loss were not markedly different from that obtained in normal pregnancy, except for the prevalence with the dRVVT test, which was more than twice that in normal pregnancy (4.3% and 10.9% respectively). It is clear that in previous studies that employed the dRVVT[12,17,23] that this test detected the presence of LA more frequently than the APTT and the KCT (Table 2). In the current study we found that the APTT, dRRVT and KCT tests had a prevalence comparable to LA in patients with RFL. However, when compared to the results obtained in normal pregnancy (Table 1), it is clear the dRVVT produced a remarkably higher rate of positive results than other tests. Of interest is that if only one or even two screening tests were performed a significant number of LA positive cases will be missed. This could make a difference

to the treating physician as to the possible etiology and further management of RFL. It is therefore advisable to routinely use the three tests (APTT, KCT and dRRVT) when screening for LA.

In the current study we followed test procedures, including the blood sampling technique and double centrifugation of the blood samples, detailed in the original assay techniques,[13] while earlier studies have undertaken extensive modification of these assay techniques. This may account for the inter-laboratory variation in antiphospholipid antibody testing, a feature that has been highlighted recently.[20] It is noteworthy that most of these earlier studies did not include the prevalence of LA in the healthy population or even in normal pregnancies, which is necessary to draw valid conclusions on the pathognomic role of these antiphospholipid antibodies in recurrent fetal loss. In one recent report[23] the prevalence of LA in patients with RFL (n=331) was 5.1% while in controls (normal pregnancy, n=993) the prevalence was 3.8%. This led the authors to conclude that there is no justification for considering LA a risk factor in women with RFL. There is yet the possibility of the existence of ethnic differences in the prevalence of these antibodies, which was not yet reported, as far as we know. Such ethnic differences were documented in a variety of haemostatic studies[24] and were closely related, for example, to the prevalence of ischaemic heart disease.[25]

The mechanism of pregnancy loss associated with antiphospholipid antibodies (APAs) remains unclear, but has consistently been centered on placental thrombosis, as explained in a recent publication.[11] On the other hand, placental thrombosis was not found in all RFL patients or in normal pregnant women with APAs. Also, RFL patients did not suffer overt systemic thrombotic disease.[11] Nonetheless, the thrombotic mechanism gained more support from the advocates of antiplatelet (aspirin) and anticoagulant (heparin) therapy[11,18,26] in the successful management of RFL patients.

In conclusion, the results of this large study have shown that if only one or even two screening tests were performed for the detection of LA, a significant number of LA-positive cases would be missed. This would make it difficult for treating physicians to make up their mind as to the possible etiology and further management of RFL. It is therefore advisable to routinely employ the three tests (APTT, KCT and dRRVT) when screening for LA.

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