The prevalence of antiphospholipid antibodies in women with reproductive failure or major abruptio placentae

Z. M. VAN DER SPUY, A. R. BIRD, S. W. LINDOW, C. BRUCE

Abstract **The so-called antiphospholipid syndrome may be responsible for repeated spontaneous abortion and fetal loss. We examined the plasma of 61 women with either reproductive failure or abruptio placentae for the presence of lupus anticoagulant and anticardiolipin antibodies in an attempt to assess the prevalence of this condition.**

No patient had haematological abnormalities consistent with the presence of lupus anticoagulant. However, 6 women had anticardiolipin antibodies with normal coagulation tests. The clinical profile together with this laboratory finding allows us to diagnose the antiphospholipid syndrome in these patients.

Our incidence is considerably lower than that reported in many other studies but does suggest a place for screening for this condition in patients with recurrent complications of pregnancy.

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The lupus anticoagulant (LA) is a spontaneously acquired anticoagulant initially described in patients with systemic lupus erythematosus (SLE).[1] It has subsequently been reported in patients with other auto-immune disorders, in association with drugs or neoplasia and in the normal population. The LA is an immunoglobulin (Ig),either IgG or IgM, that acts by inhibiting phospholipid activity in clotting reactions. Characteristically, the anticoagulant prolongs the activated partial thromboplastin time (APTT) and fails to correct upon mixing with equal volumes of normal plasma. The prothrombin time (PTT) is only slightly prolonged when measured with undiluted thromboplastin.[2]

The LA is not associated with a haemorrhagic tendency, unless there is a concomitant qualitative or quantitative platelet or clotting factor abnormality.[3] Paradoxically, an increase in thrombo-embolic events and a high rate of spontaneous fetal loss have been observed in patients with this laboratory abnormality.[4-9] This is particularly so in patients with SLE and LA.

There have been a number of reports of the presence of LA or anticardiolipin antibodies or both in women who have suffered repeated spontaneous abortion and fetal loss. The prevalence of antiphospholipid antibodies in this group of patients varies considerably in the different studies and ranges from 7% to 50%.[10] Because therapy with aspirin and glucocorticoids is often successful in treating this condition, it has been recommended that all women presenting with reproductive failure should be screened for LA and anticardiolipin antibodies.[11]

In order to study this condition in our patient population we examined plasma samples for the presence of LA and anticardiolipin antibodies in women with a history of reproductive failure (recurrent spontaneous abortions, unexplained stillbirth or intra-uterine growth retardation) and women who had suffered severe abruptio placentae.

Departments of Obstetrics and Gynaecology and Haematology, Groote Schuur Hospital and University of Cape Town
Z. M. VAN DER SPUY, M.B. CH.B., PH.D., F.R.C.O.G.
A. R. BIRD, M.B. CH.B., M.MED. (PATH.)
S. W. LINDOW, M.MED., F.C.O.G., M.R.C.O.G.
C. BRUCE, M.B. CH.B.

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Patients and methods

Consent to perform this study was obtained from the Ethics and Research Committee of the University of Cape Town Medical School. Informed consent was given by all patients and controls studied.

Patients

Three groups of women with no clinical evidence of SLE-related collagen disorders or other illnesses known to cause abnormalities in haemostasis tests were investigated.

Abruptio placentae

Thirty women with a history of major abruptio placentae in a recent pregnancy were studied. They were recalled for assessment 1 - 3 years after the index pregnancy and there was thus no likelihood of the coagulation problems associated with abruptio placentae affecting the laboratory studies.

Reproductive failure

Thirty-one women attending the reproductive failure clinic at Groote Schuur Hospital were included in this study. Women attending this clinic had all had 2 or more spontaneous abortions and possibly other forms of reproductive failure such as unexplained stillbirth or severe intra-uterine growth retardation. The subjects studied had been fully investigated and no cause for their reproductive problems had been found. All serological tests for syphilis were negative.

Control subjects

A group of 20 normal adult women and 20 normal adult men served as the control group to establish the reference range for the tests described below, which were used to detect the LA.

Laboratory methods

Full blood and platelet counts were performed on all patients. PTT, APTT, kaolin clotting time (KCT), Russell viper venom time (RVVT) were measured in all the subjects, who were also assessed for the presence of anticardiolipin antibodies.

Blood was taken by venepuncture into a 0,1 volume of 3,8% trisodium citrate. Platelet-rich plasma (PRP) was prepared by centrifuging of blood at 200 *g* for 20 minutes at room temperature. Platelet-poor plasma (PPP) was prepared by centrifuging of the blood at 3 000 *g* for 15 minutes at room temperature. The PPP was further filtered through 0,22 μm cellulose acetate filters in stainless steel assemblies (Millipore Filter, type G5), in order to extract as many platelets as possible; this reduced the platelet phospholipid content in an attempt to enhance the sensitivity for the LA.[12] All haemostatic tests were performed by means of both filtered and unfiltered plasma. The APTT was carried out by means of the Automated APTT Reagent on the

Coag-a-mate X2 (General Diagnostics, New Jersey). The PTT was calculated on the Clotek II (Hyland) and the Automated Coagulation Laboratory (Instrumentation Laboratories, Milan, Italy) by means of undiluted acetone-dried human brain thromboplastin.[13] The KCT was measured as previously described and the dilute RVVT was assessed according to the method of Thiagarajan *et al.*[14] Enzyme-linked immunosorbent assay (ELISA) was used to detect plasma cardiolipin antibodies (personal communication — R. Cooper).

Results

Abruptio placentae group

The age range of these patients was 17 - 38 years. In 28 of the 30 women a retroplacental clot had been found at delivery. Nine infants survived, 2 suffered early neonatal deaths and the remaining 19 were stillborn. The birthweight varied from 550 g to 4 600 g and the gestational age from 24 weeks to 40 weeks with a mean (± SD) of 34, 7 ± 4 weeks. Twenty-five of the patients were multiparous and the remaining 5 were primigravidas. Of the 30 women, 15 were hypertensive either before or after their delivery and 14 had proteinuria. The records of 2 patients' postpartum blood pressure were missing.

Reproductive failure group

The age range of the patients was 19 - 33 years. The 31 women studied had had a total of 157 pregnancies. Twenty-three were pregnant at the time of the study. The remaining 135 pregnancies had resulted in 23 livebirths (5 of these infants had been severely growth retarded), 13 stillbirths, 3 ectopic pregnancies and 96 abortions. Eighty of these abortions had occurred at less than 12 weeks, 13 at 13 - 20 weeks and the remaining 3 at 21 - 28 weeks. In addition there had been 4 neonatal deaths and 2 paediatric deaths among the liveborn infants.

Laboratory results

The full blood and platelet counts were within normal ranges in all the women. The means and ranges of the results of haematological tests in the control group are outlined in Table I. The means for all the tests, apart from the PTTs, were significantly greater with filtered plasma. The assay for cardiolipin antibodies was considered positive when more than 5 units of IgG antibodies were found. Since practically all the assays were single, as opposed to serial, tests, IgM results were ignored in cases of nonspecific cross-reactions with other antibodies. In the spontaneous abortion group only 1 of the 30 patients had a result outside the range of the control group in more than one coagulation test, namely an isolated mild prolongation of the APTT in both filtered and unfiltered PPP, which corrected to within 4 seconds of the control with a 1:1 mixture of normal plasma. Assays of factor VIII, IX, XI and XII were normal with no dilutional effect. The results for the two groups of patients are summarised in Table II.

TABLE II.
Numbers of normal and abnormal laboratory results in patients

	Abruptio placentae group	Reproductive failure group
No. of patients	30	31
Patients with all results normal	26	25
Patients with abnormal results	4	6
Abnormal results		
APTT	0	1*
Filtered APTT	1	2*
RVVT	0	1
Anti-cardiolipin antibodies	3	3
Collagen screen	0	0

* 1 patient had 2 abnormal results.
APTT = acitvated partial thromboplastin time.
RVVT = Russell viper venom time.

Two women in the reproductive failure group had isolated results (< 1,0 seconds) outside the control group reference values for the filtered APPT and RVVT respectively, while 1 patient in the abruptio placentae group had a similar mild prolongation of the filtered APTT test. All these corrected fully after administration of a 1:1 mixture with normal plasma. Three women in each group had anticardiolipin antibodies, but none of these women had any coagulation test abnormalities. Collagen vascular screens were performed on all the patients with reproductive failure and none had serological evidence to suggest an underlying auto-immune disease.

In the reproductive failure group, women who had undergone a positive serological test for syphilis or who had a known cause for abortion were excluded from this study. Of the 3 women with positive anticardiolipin antibodies, 2 had had 3 and 8 abortions respectively and no successful pregnancies; the third woman had delivered 2 live healthy children and had subsequently had 3 abortions.

The 3 women with abruptio placentae who were found to have anticardiolipin antibodies had negative results on serological testing for syphilis. The abruptio placentae occurred in their first, third and seventh pregnancies respectively; all the babies were stillborn and weighed 700, 1 700 and 4 000 g respectively. The woman in her seventh pregnancy had also had a previous stillbirth of unknown cause.

Discussion

Six of our patients had positive anticardiolipin antibodies. However, despite the use of a wide battery of coagulation screening tests, none of the women studied had evidence to suggest the presence of LA, namely: (*i*) prolongation of the APTT, KCT or simlar test; (*ii*) failure of 1:1 mixture with normal plasma to correct

TABLE I.
Means and ranges for the screening tests in 40 control subjects*

	APPT (seconds)		KCT (seconds)		RVVT (seconds)	
	Pre-filter	Post-filter	Pre-filter	Post-filter	Pre-filter	Post-filter
Mean ± SD	29,8 ± 2,9	31,7 ± 3,3	80,9 ± 13,2	145,7 ± 34,3	28,6 ± 2,6	29,4 ± 2,4
Reference range (± SD)	24 – 35,6	25,1 – 38,3	54,5 – 107,3	77,1 – 214,3	23,4 – 33,8	24,4 – 34,4
Comparison of pre- and post-filter results (paired *t*-test)	$P < 0,0001$		$P < 0,0001$		$P < 0,001$	

* The mean and range for the prothrombin time was 13,7 s and 12,6 - 15,2 s respectively — no significant difference was seen between filtered and unfiltered plasma.

a prolonged APTT to within 4 seconds of the control; (*iii*) decrease in at least two factors (VIII, IX, XI or XII) by one-stage assay; and (*iv*) a sensitivity to dilution.[15] One patient with a clearly prolonged APTT corrected on a 1:1 normal plasma mix and had normal levels of the intrinsic pathway factors. We have not been able to obtain further follow-up specimens and can therefore only surmise that she may have had a deficiency of one of the other factors involved in contact activation, e.g. high molecular weight kininogen. The 3 other patients had isolated mild abnormalities (< 1,0 second outside control reference values) which similarly did not fulfil the criteria for the LA, and probably represent a normal level just outside the 95% confidence limits used for the reference values.

Harris[10] has suggested guidelines for the diagnosis of 'antiphospholipid' syndrome. Patients should have at least one clinical and one serological feature of this syndrome at some time in their disease course. The clinical features mentioned by Harris are venous thrombosis, arterial thrombosis and recurrent fetal loss, while the three serological findings of importance are IgG anticardiolipin antibodies, positive LA tests and IgM anticardiolipin antibody plus positive LA test.[10,16] According to these criteria, 6 of our patients had the antiphospholipid syndrome.

Our finding of a low incidence of antiphospholipid syndrome in this group of women with pregnancy loss is contrary to the results of most previous studies, in which the incidence ranged from 7% to 51,6%.[10,11,17] It is unlikely that our coagulation screening tests are less sensitive than those in previous studies since we used a wide spectrum of tests previously reported to be sensitive to the LA. As mentioned earlier, in an attempt to heighten the sensitivity we filtered the PPP to remove as many platelets as possible, thereby reducing to a minimum the masking effect of platelet phospholipids.[12]

Although the findings in our study demonstrate a considerably lower incidence of anticardiolipin antibodies than usual in a group of women with abruptio placentae or recurrent fetal loss, the incidence is just under 10% and we suggest that it is advisable to screen patients who present with fetal loss or a previous history of abruptio placentae.

Lubbe *et al.*[18] and Pattison *et al.*[19] originally described the presence of the LA ± anticardiolipin antibodies in women with recurrent fetal loss. They successfully treated these patients with low-dose aspirin and immunosuppressive doses of corticosteroids. The aim of their therapy was to normalise the clotting factors and many of their patients subsequently delivered live healthy children.[20] A very high prevalence of antiphospholipid antibodies in patients with high fetal wastage — recurrent abortion, growth retardation and unexplained stillbirths was, however, reported.[11,19] Other workers have confirmed their work. Howard *et al.*[17] reported a 48% prevalence of LA in women with recurrent miscarriage who did not have SLE. In another study, Deleze *et al.*[21] studied patients with SLE who had been investigated for recurrent fetal loss and compared them with healthy women who had suffered recurrent abortions and a group of healthy women who had had at least 3 successful pregnancies. The prevalence of antiphospholipid antibodies was high in the patients with SLE and just under 10% in the women with recurrent fetal loss. None of the women with a normal obstetric history had evidence of these antibodies. The prevalence in their study therefore is in the same range as ours.

In conclusion, there have been reports of vasculopathies, placental infarction and the development of hypertension in pregnancy in patients who have antiphospholipid antibodies.[22-24] Recurrent early pregnancy loss is possibly just one aspect of pregnancy compromise in these patients and we should be alert to the possible association of the antiphospholipid syndrome and other pregnancy complications.

Given the ease of therapy and the good results which have been reported to date, we suggest that patients who present with recurrent fetal loss, unexplained stillbirth, previous abruptio placentae or gestational proteinuric hypertension should be screened for antiphospholipid antibodies and that, where appropriate, aspirin and possible steroid therapy should be initiated. It should be noted however, that in some women these antibodies only appear during pregnancy and disappear thereafter. A negative result in the non-pregnant patient needs further follow-up during pregnancy and patients should be appropriately counselled.

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