Methods for detecting lupus anticoagulants and their relation to thrombosis and miscarriage in patients with systemic lupus erythematosus

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Abstract

Aims: To examine the sensitivity and specificity to past thrombotic events of four different coagulation tests, which screen for lupus anticoagulant (LA), and of anticardiolipin antibodies in patients with systemic lupus erythematosus.

Methods: Fifty three consecutive patients with systemic lupus erythematosus were studied of whom three males and 21 females, aged 21-60 years, had a history of venous and arterial thrombosis, or miscarriage, or both. Activated partial thromboplastin time (aPTT), dilute Russell's viper venom time (dRVVT), kaolin clotting time (KCT), dilute aPTT and the circulating titre of anticardiolipin antibodies were investigated in the two groups of patients and in 20 healthy control subjects.

Results: The prolonged dilute aPTT was more sensitive to thromboses or miscarriages, or both than dRVVT ($p < 0.05$), KCT ($p < 0.01$), and aPTT ($p < 0.001$). No significant differences in specificity were found among aPTT (100%), dRVVT (93%), KCT (93%) and dilute aPTT (86.2%); but aPTT and dRVVT were significantly more specific ($p < 0.01$, $p < 0.05$, respectively) than anticardiolipin antibodies.

Conclusions: The study shows a strong association between lupus anticoagulant and thrombosis when a very sensitive test such as the dilute aPTT is used. The combination of this assay with a very specific test such as dRVVT might enable patients with SLE at high risk of thrombosis to be identified.

The lupus anticoagulant is an antibody (usually IgG, IgM, or a mixture of both) that is directed against the negatively charged phospholipids of the prothrombin activator complex.¹² It therefore prolongs in vitro the phospholipid dependent coagulation tests without inhibiting the activity of specific coagulation factors.³

Although lupus anticoagulant may be seen in several clinical conditions, in association with drugs regimens, following viral infections including AIDS, in haematological malignant diseases, and in apparently healthy subjects,⁴⁻⁶ it was initially described in autoimmune disease and, in particular, in

patients with systemic lupus erythematosus (SLE).⁷

Previous reports have suggested the prevalence of lupus anticoagulant in patients with SLE to be between 10% and 15%,⁸⁻¹⁰ but other recent studies have found the prevalence to be 21 to 65%.¹¹⁻¹³ This discrepancy may depend on the fluctuation of lupus anticoagulant due to disease activity or treatment,^{14 15} but it is more probably due to the different sensitivities of the various tests and reagents used to verify the presence of lupus anticoagulant.¹⁶⁻¹⁸ More recently, some authors have also suggested possible laboratory heterogeneity among "lupus anticoagulants".¹⁹⁻²¹

The routinely used coagulation tests in which phospholipids are added to the test system, especially activated partial thromboplastin time (aPTT), have been found by different authors to be insufficiently sensitive to the presence of lupus anticoagulant.^{22 23} The reduction or the elimination of exogenous phospholipids in the dilute Russell's viper venom time (dRVVT) and kaolin clotting time (KCT), respectively, actually increased the sensitivity to lupus anticoagulant.^{12 24} The positivity of lupus anticoagulant is associated with a tendency to thromboembolic events; thrombosis is common in patients with lupus anticoagulant, where it ranges from 25%-35%,²⁵ although some authors have found a higher prevalence.^{7 11 26} Lupus anticoagulant positive women also have a high risk of recurrent abortions due to thrombosis of the placental and decidual vessels.^{27 28}

The variability in the prevalence of thrombotic phenomena in such patients could depend on the differing capacity of the tests to detect lupus anticoagulant. In fact, it seems that a stronger association can be found between lupus anticoagulant and thrombosis using tests which are more sensitive to and specific for the presence of lupus anticoagulant.^{22 29 30}

The aim of this study was to compare the respective sensitivity and specificity to thrombotic episodes of four coagulation tests (aPTT, dRVVT, KCT, dilute aPTT), previously shown to have varying capacities to detect lupus anticoagulant.^{12 22 24 31} Furthermore, we evaluated the titre of circulating anticardiolipin antibodies which have been reported as being strongly associated with lupus anticoagulant^{32 33} and measurable by several immunoassays.^{34 35}

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Accepted for publication
5 September 1991

Methods

The study was carried out on 53 consecutive patients (eight males, 45 females, aged 16–60 years) with SLE defined according to the American Rheumatism Association's criteria.³⁶ They were examined in our Institute between January 1988 and June 1990 after informed written consent had been obtained. Twenty four of these patients (three males, 21 females, aged 21–60 years) had a history of venous and arterial thrombosis or miscarriages occurring over the previous one to 10 years. In the thrombotic patient group the average duration of SLE was seven years (range two to 11). Twenty nine patients (five males, 24 females, aged 16–58 years) had no history of thromboses or miscarriages. In these patients the clinical history of SLE had begun, on average, eight years previously (range two to 19 years). The two groups of patients were similar with respect to age, sex, and disease activity. Forty four patients (21 in the group with a history of thrombosis, 23 in the group without thrombotic episodes) were taking prednisone (12.5–50 mg/day), methylprednisolone (4–24 mg/day), or methotrexate (20 mg weekly). None of the patients under examination had undergone anticoagulant, antiaggregant, or fibrinolytic treatment during the month before the study. Blood samples collected in one tenth volume of sodium citrate (0.13 M), were taken from patients after a 12 hour fast. Platelet poor plasma (PPP) was obtained by double high speed centrifugation at $5000 \times g$ for 10 minutes at room temperature. Four different coagulation tests were used: aPTT, dRVVT, KCT and dilute aPTT. All patient samples giving abnormal results were re-performed by mixing fresh patient's plasma in a 1:1 ratio with fresh pooled normal plasma, to allow for replacement of any factory deficiency. If the time remained prolonged the test was validated as abnormal. The *activated PTT* was carried out by incubating, for three minutes at 37°C, 0.1 ml of plasma sample with 0.1 ml of standard phospholipid mixture and activator (Activated Thromboplastin, Ortho Diagnostics; Milan); 0.1 ml of 0.025 M of calcium chloride was then added and the clotting time recorded on a Schnitger und Gross coagulometer.²⁹ aPTT was expressed as patient:normal ratio (normal values of ≤ 1.2).

The *dilute Russell's viper venom time* (dRVVT) was carried out by incubating, for three minutes at 37°C, 0.1 ml of Russell's viper venom (Wellcome Diagnostics, Dartford, England) diluted 1 in 200 in TRIS buffered saline (0.15 mol/l sodium chloride, 0.02 mol/l TRIS, pH 7.5), 0.1 ml of diluted phospholipid reagent (Thromboplastin, Ortho Diagnostics, Milan; diluted 1 in 8 in TRIS-buffered saline), and 0.1 ml of plasma sample. Clotting time was recorded after the addition of 0.1 ml of 0.025 M calcium chloride.²⁴ The normal range, determined on 20 fresh normal plasmas (20 healthy subjects, five males, 15 females, aged 20–58 years) was 26.4 (SD 1.4) seconds. Clotting time longer than 30 seconds (mean + 2.5 SD of controls) was considered to be abnormal.

Kaolin clotting time, originally described by Margolis,³⁷ was measured by incubating, for two minutes at 37°C, 0.05 ml of 2% diluted kaolin with 0.1 ml of plasma sample; clotting time was recorded by adding 0.1 ml of 0.025 M calcium chloride.¹² The normal range, determined on 20 fresh normal plasmas, was 48.8 (SD 8.0) seconds. Clotting time of 65 seconds or greater (mean + 2 SD of controls) was considered to be abnormal.

The *dilute aPTT*, assessed by serial phospholipid dilutions, was performed as follows: 0.1 ml of plasma sample was incubated for three minutes at 37°C with 0.1 ml of kaolin suspension diluted 1 in 15 (Boehringer Mannheim, Germany) and 0.1 ml of Thromboplastin (Ortho Diagnostics; Milan) diluted in two different ratios (1:5 and 1:80) with veronal buffer at pH 7.4. Clotting time was recorded after the addition of 0.1 ml of 0.025 M calcium chloride.³⁸ In the 20 healthy subjects the difference between 1 in 80 and 1 in 5 Thromboplastin dilutions was 9 (SD 2.8) seconds.

Dilute aPTT was considered abnormal if the difference between 1 in 80 and 1 in 5 Thromboplastin dilutions was more than 15 seconds (mean + 2 SD of controls). To confirm if the abnormality of all the phospholipid dependent tests was due to the presence of lupus anticoagulant, 0.1 ml of 0.05 mM phosphatidylserine-phosphatidylcholine (PS-PC) liposomes were added to the plasma phospholipid mixture to neutralise the anticoagulant effect of antiphospholipid antibodies, as previously described.³⁹

For the evaluation of the anticardiolipin antibodies an enzyme linked immunosorbent assay (ELISA) was used. This method was validated in an international workshop and is outlined below.⁴⁰ Briefly, 95-well, flat-bottomed, rigid polystyrene electroimmunoassay microtitration plates (Dynatech) are coated (30 μ l/well) with bovine heart cardiolipin (Sigma Chemical Company, St Louis, Missouri) diluted in ethanol. The ethanol is evaporated by leaving the plates uncovered overnight at 4°C. After the plates have been dried they are washed twice with phosphate buffered saline (pH 7.3) 100 μ l/well. The plates are blocked (75 μ l/well) with 10% fetal calf serum in phosphate buffered saline for one hour. Aliquots (50 μ l) of normal human serum, positive control samples, and test samples at 1 in 50 dilution in phosphate buffered saline-fetal calf serum (10%) are then added to triplicate wells and incubated for three hours at room temperature. The plates are then washed three times with phosphate buffered saline, and alkaline phosphatase labelled, affinity isolated, goat antihuman IgG or IgM, or both, diluted 1 in 1000 in phosphate buffered saline-fetal calf serum is added (50 μ l/well). Plates are incubated for 30 minutes at room temperature. The conjugate is discarded and the plates washed three times with phosphate buffered saline. Substrate (50 μ l/well) is then added. The substrate is prepared by adding one tablet of p-nitrophenyl phosphate (Sigma) per 5–6 ml diethanolamine buffer. After addition of substrate the reaction is allowed to take place in the

dark at 37°C, and when the highest positive control value reaches an absorbance reading of about 0.8 to 0.9 for both the IgG and IgM samples, the reaction is stopped by adding 3N sodium hydroxide 75 µl/well. Absorbance is read at 405 nm using a multiscanner. The results are expressed as the number of standard deviations above the mean in normal human serum (reference value <3 SD). Finally, the possible presence of intravascular clotting activation was investigated in all patients by measurement of D-Dimer (Ortho Dimertest, Ortho Diagnostics Systems; Beerse, Belgium; reference value of <200 ng/ml), a marker of *in vivo* fibrinolysis.⁴¹

Statistical analysis of data was performed by binomial probability distribution (two tailed exact test).⁴² The differences in sensitivity and specificity were assessed by the χ^2 test and Fischer's exact test for independence. The measures of association of anticardiolipin antibody subclasses and aPTT, KCT, dRVVT and dilute aPTT were taken as continuous values and evaluated by the Kendall τ correlation test.

Results

Among the 53 patients with SLE, 24 (45%) had one or more clinical events related to thrombotic phenomena: nine (17%) subjects had thrombophlebitis, five (9%) had had a stroke, and 14 (26%) in whom obstetric pathology was excluded, had miscarriages in the first or second trimester; four patients had more than one clinical event (table 1). Twenty nine patients (55%) had no history of thromboses or miscarriages. Among all patients, 23 (43%) had a prolonged dilute aPTT, 14 (26%) prolonged dRVVT, 13 (24.5%) prolonged KCT, five

(9.5%) prolonged aPTT and 22 (41%) showed increased titres of circulating anticardiolipin antibodies.

In patients with a history of thrombosis or miscarriage the dilute aPTT was prolonged in 19 subjects (79%), while dRVVT was abnormal in 12 cases (50%). In all patients with prolonged dilute aPTT and dRVVT the confirmatory procedure with 0.05 mM PS/PC liposomes normalised the coagulation time (data not shown). KCT and aPTT were prolonged in 11 (46%) and five (21%) patients, respectively. In the same subgroup the anticardiolipin antibody titres were increased in 13 (54%) subjects (table 1).

Among the 29 patients without a history of thrombosis or miscarriages, a prolonged dilute aPTT was found in four subjects, while dRVVT and KCT were abnormal in two patients. In two of these subjects with prolonged diluted aPTT and dRVVT the confirmatory test with PS/PC liposomes corrected the coagulation time (data not shown). In this subgroup there were no patients with prolonged aPTT, but nine subjects with SLE without thrombotic episodes showed high circulating titres of anticardiolipin antibodies (table 2).

In all patients the high concentrations of anticardiolipin antibodies were especially dependent on the presence of high titres of circulating anticardiolipin IgG antibodies, which were found in 86.4% compared with 27.3% of anticardiolipin IgM antibodies and 22.7% of anticardiolipin IgA antibodies (tables 1 and 2).

The sensitivity and the specificity of the dilute aPTT, dRVVT, KCT, aPTT and anticardiolipin antibody titres for thrombotic

Table 1 aPTT, KCT, dRVVT, dilute aPTT and anticardiolipin antibodies in patients with thrombosis, miscarriage, or both

Case No	aPTT (ratio)	Kaolin clotting time (sec)	Dilute Russell's viper venom time (sec)	Dilute aPTT (sec)	Anticardiolipin antibodies (SD)	Clinical events
1	1.08	60	28	18*	IgG 46 IgM 6 IgA 5.6	6 miscarriages in the first and second trimester; 1 live birth
2	0.88	78	37.8*	20*		1 stroke
3	1.11	90*	29	27*	IgG 38 IgM 16	2 miscarriages in the first and second trimester; no live births; 1 thrombophlebitis
4	1.39*	80*	31*	19*		2 miscarriages in the second trimester; no live births
5	1.08	80.2*	30.6*	22*	IgM 4.4 IgG 11.2 IgM 14.4	Recurrent thrombophlebitis
6	0.92	53.7	29	13		2 miscarriages in the second trimester; no live births
7	1.04	63.9	29.1	22*	IgG 17.4 IgG 12.4 IgG 4	2 miscarriages in the second trimester; no live births
8	1.04	63	32*	14		2 miscarriages in the second trimester; no live births
9	0.96	65*	32*	17*	IgG 6.6 IgG 12.7	5 miscarriages in the first and second trimester; 1 live birth
10	1.36*	78.7*	37.2*	27*		1 stroke; 1 thrombophlebitis
11	1.04	71*	29	21.4*	IgG 10 IgM 14.4 IgA 3.6	1 miscarriage in the second trimester; no live births
12	1.24*	65.9*	29.9	27*		1 stroke
13	1.08	57.9	24.9	6	IgG 15 IgA 18 IgG 11.6 IgG 4.4	1 stroke
14	0.96	40.6	30.5*	27.2*		3 miscarriages; 2 in the second trimester. 1 in the first; no live births
15	1.22*	55.2	33.1*	17*	IgG 18 IgG 11.6 IgG 4.4	1 thrombophlebitis
16	1.52*	78.4*	32.7*	18*		1 thrombophlebitis
17	0.88	49.5	26	11	IgG 10 IgM 14.4 IgA 3.6	2 miscarriages in the first and second trimester; no live births
18	0.90	42.8	22.9	7		1 thrombophlebitis
19	1.02	48.2	33.4*	22*		3 miscarriages in the first and second trimester; no live births; 1 thrombophlebitis
20	0.92	49.9	26	16*	IgG 15 IgA 18 IgG 11.6 IgG 4.4	2 miscarriages in the second trimester; no live births
21	1.18	81.9*	31*	17.3*		4 miscarriages in the first and second trimester; 1 live birth; 1 thrombophlebitis
22	1.04	66*	30*	15.5*	IgG 18 IgG 11.6 IgG 4.4	1 stroke
23	1.08	47.7	27.3	19*		1 thrombophlebitis
24	1.04	63.9	29.1	22*		1 miscarriage in the second trimester; no live births

*Abnormal values.

Table 2 aPTT, KCT, dRVVT, dilute aPTT and anticardiolipin antibodies in patients with thrombosis or miscarriage

Case No	aPTT (ratio)	Kaolin clotting time (sec)	Dilute Russell's viper venom time (sec)	Dilute aPTT (sec)	Anticardiolipin antibodies (SD)
1	1.0	55	28	12.7	
2	1.08	54	34.2*	24.5*	IgG 26 IgM 6.8 IgA 2.8
3	0.92	58.7	24	10	
4	1.04	53.8	28.9	11	
5	1.08	46.7	28.3	11	IgG 24.5
6	1.12	52.6	28.7	16*	
7	1.08	55.9	29.2	11.4	IgG 4.4 IgM 26
8	0.88	46.8	24	9	
9	1.08	78*	32*	27*	
10	1.0	51.2	25	11	
11	1.0	48.2	25.6	11	
12	1.04	55.9	27.4	13	
13	1.06	61	27.7	14	IgG 9.6 IgG 6.5
14	0.92	49	25	12	
15	0.96	61	28	10	
16	1.04	65*	26	12	
17	1.0	49	24	10	
18	0.96	57.4	24	9	
19	0.96	45.6	23.6	11.5	
20	1.02	48.6	25.4	14	IgG 3.5 IgA 4
21	1.12	58.2	28	12.8	
22	1.01	56.6	27.3	29*	
23	1.10	62.1	24.6	4	
24	0.80	44.4	22.4	11	
25	1.04	49.3	27.1	7	
26	1.0	45.3	25.6	12	
27	0.88	56.4	25.8	15	IgG 12.25
28	0.92	58.9	28	11.5	
29	1.08	43.1	26.5	6	

*Abnormal values.

events and miscarriages are shown in table 3.

The prolonged dilute aPTT was more sensitive than the dRVVT (79% *v* 50%; $p < 0.05$), KCT (79% *v* 45.8%; $p < 0.01$), aPTT (79% *v* 20.8%; $p < 0.001$). No significant differences were found between dilute aPTT and anticardiolipin antibody titres.

The dRVVT, KCT, and anticardiolipin antibodies antibody titres were more sensitive than aPTT (50% *v* 20.8%, $p < 0.05$; 45.8% *v*

Table 3 Sensitivity and specificity to thrombosis and miscarriages of aPTT, dilute aPTT, dRVVT, KCT and anticardiolipin antibodies

Assays	Sensitivity (95% confidence intervals) $n = 24$ %	Specificity (95% confidence intervals) $n = 29$ %	†p Value
Dilute aPTT	79 (19/24) (57.85–92.87)	86.2 (25/29) (71.77–97.73)	<0.001
dRVVT	50 (12/24) (29.12–70.88)	93 (27/29) (81.65–99.91)	<0.01
KCT	45.83 (11/24) (25.5–67.18)	93 (27/29) (81.65–99.91)	<0.01
aPTT	20.8 (5/24) (7.13–42.15)	100 (29/29) (87.66–100)	<0.02
Anticardiolipin antibodies	54 (13/24) (32.82–74.45)	69 (20/29) (49.17–84.72)	NS
Differences:			
Dilute aPTT, dRVVT	29* (10–47.5)	6.8 (–2–16)	
Dilute aPTT, KCT	33.2** (13.9–52.4)	6.8 (–2–16)	
Dilute aPTT, aPTT	58.2*** (38.1–78.3)	13.8 (1–26.6)	
Dilute aPTT, anticardiolipin antibodies	25 (7.3–42.6)	17.2 (3–31.2)	
KCT, aPTT	25* (7.3–42.6)	7 (–2.5–16)	
dRVVT, aPTT	29.2* (10.6–47.7)	7 (–2.5–16)	
dRVVT, KCT	4.2 (–4–12.4)	0 (0–0)	
Anticardiolipin antibodies, aPTT	25.2* (7.47–43)	31** (13.8–48.2)	
Anticardiolipin antibodies, dRVVT	4 (–4–12)	24* (8.1–39.9)	
Anticardiolipin antibodies, KCT	8.2 (–3–19.4)	24 (8.1–39.9)	

Parentheses contain 95% confidence intervals calculated with the binomial distribution for sensitivities and specificities and with normal approximation for differences.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.† χ^2 with continuity correction or (if $n < 5$) Fisher's exact test.

20.8%, $p < 0.05$; 54% *v* 20.8%, $p < 0.05$, respectively). No significant differences in sensitivity were found between the dRVVT, KCT, and anticardiolipin antibodies titres (table 3).

aPTT was more specific to thrombotic events and miscarriages than dilute aPTT, dRVVT, and KCT, but the differences were not significant. aPTT and dRVVT, on the contrary, were significantly more specific than anticardiolipin antibody titres ($p < 0.01$; $p < 0.05$, respectively) (table 3).

The analysis of cross classification data shows a significant association between thrombotic episodes or miscarriages, or both, and the abnormality of dilute aPTT ($p < 0.0001$), dRVVT ($p < 0.01$), KCT ($p < 0.01$) and aPTT ($p < 0.02$). No significant association was found between clinical events and anticardiolipin antibody titres (table 3).

In all patients with presumed thrombotic events further analysis was carried out distinguishing patients with arterial and venous thrombosis from those with miscarriages alone. In the group of patients ($n = 13$) with arterial and venous thromboses we found a significant correlation between clinical events and abnormal dilute aPTT ($p < 0.0001$), dRVVT ($p < 0.001$), KCT ($p < 0.001$), aPTT ($p < 0.01$), and anticardiolipin antibody titres ($p < 0.05$), respectively (table 4).

In the group of patients with miscarriages alone we found a significant association between clinical events, dilute aPTT ($p < 0.001$) and dRVVT ($p < 0.05$) (table 5).

The correlation between laboratory tests that screen for lupus anticoagulant and anticardiolipin antibody titres was also studied. Dilute aPTT and dRVVT were the only tests that significantly correlated with anticardiolipin antibodies, in particular with anticardiolipin IgG titres (table 6).

In all patients, the evaluation of circulating concentrations of D-Dimer showed normal values (< 200 ng/ml), thus precluding intravascular clotting action.

Discussion

The natural history of patients with SLE can be complicated by thromboembolic accidents which range from 4.5–18% of cases,^{43–46} while miscarriages have been reported to occur in 16% of such patients.⁴⁷ Other studies, however, have shown a higher incidence of clinical events related to thrombosis in SLE.^{11,48}

A relatively high rate of arterial and venous thrombosis (24%) and miscarriages (26%) was found in our patients with SLE; this might be explained by a high number of lupus anticoagulant positive patients. A large variability in lupus anticoagulant positivity was related to the tests used. In fact the prevalence of lupus anticoagulant, diagnosed the revised scientific subcommittee criteria for lupus anticoagulants,⁴⁹ ranged from 43%, when assessed by dilute aPTT, to 9.5% when assessed by aPTT. This variable capacity to detect the presence of lupus anticoagulant probably

Table 4 Association between venous and arterial thrombosis and abnormal laboratory tests in patients with SLE

Abnormal assays	Patients with SLE arterial and venous thrombosis n = 13	Patients with SLE without thrombosis n = 29	χ^2 test with continuity correction or Fisher's exact test p Value
aPTT	4 (31%)	0 (0%)	<0.01
dRVVT	8 (62%)	2 (7%)	<0.001
KCT	8 (62%)	2 (7%)	<0.001
Dilute aPTT	11 (85%)	4 (14%)	<0.0001
Anticardiolipin antibodies	9 (69%)	9 (31%)	<0.05

Table 5 Association between miscarriages and abnormal laboratory tests in patients with SLE

Abnormal assays	Patients with SLE and miscarriage only n = 11	Patients with SLE and without thrombosis n = 29	χ^2 test with continuity correction or Fisher's exact test p Value
aPTT	1 (9%)	0 (0%)	<NS
dRVVT	4 (36%)	2 (7%)	<0.05
KCT	3 (27%)	2 (7%)	<NS
Dilute aPTT	8 (73%)	4 (14%)	<0.001
Anticardiolipin antibodies	4 (36%)	9 (31%)	<NS

explains the different associations found between lupus anticoagulant tests and thrombosis in our patients. In fact, dilute aPTT showed a higher sensitivity to thrombotic events, compared with aPTT, KCT, and dRVVT.

Currently, the aPTT is the test usually performed when screening for lupus anticoagulant. When the test was prolonged, the activity of clotting factors was normal and when the addition of an equal part of normal plasma did not normalise the coagulation time, the presence of lupus anticoagulant was suspected.⁹ In previous studies this assay showed poor sensitivity to lupus anticoagulant and did not correlate with thrombotic events in patients with SLE.²²⁻²⁹ The sensitivity of the aPTT to lupus anticoagulant, however, varies depending on the different reagents and test system used.¹⁶ In fact, some aPTT reagents and methods were shown to be very sensitive to lupus anticoagulant and these differences seem to be particularly the result of the nature and quantity of the phospholipid used as a platelet substitute.¹⁷⁻⁵⁰⁻⁵¹ The low sensitivity of the aPTT observed in our study may also be attributed to a poor sensitivity to lupus anticoagulant of phospholipid preparations used in the test system.

The KCT has been found to have good sensitivity to lupus anticoagulant in several recent studies,¹⁸⁻⁵² and it seems particularly useful in screening plasma samples from pregnant women.²⁸ KCT also utilises platelet poor plasma with no exogenous phospholipids and is then sensitive to residual platelets.⁵³ In our study we found the KCT normal values to be shorter than those found by other authors.²¹⁻⁵² Although PPP was obtained by double high

speed centrifugation at 5000 $\times g$ for 10 minutes, this suggests that platelet contamination may account for the rather poor performance of this test in our study. Consequently it might be critical to remove platelets completely from plasma by a filtration step, but this may be difficult to adapt for routine screening.

The dRVVT is a coagulation test for lupus anticoagulant, based on a modified Russell's viper venom time, using limited amounts of phospholipid and venom. The dRVVT is insensitive to specific factor deficiencies caused by antibodies to factors VIII, IX, and XI.²⁴ Some authors showed that this test was more sensitive than aPTT and tissue thromboplastin inhibition procedure (TTI).²⁴ It should therefore be very useful as a screening test for lupus anticoagulant. dRVVT was also described as significantly associated with past thrombotic events, like stroke and deep venous thrombosis, in patients with SLE, while no association with miscarriages was detected.²² Recent preliminary data have suggested that the sensitivity of dRVVT to lupus anticoagulant is highly variable, depending on the selection of the phospholipid for the assay.⁵⁴

Dilute aPTT, modified by Alving,³¹ utilises several dilutions of exogenous phospholipids added to coagulation system.³⁹ Dilute aPTT can show that the circulating inhibitor is directed against negatively charged phospholipids. This is shown by the inverse correlation between the phospholipid content of the coagulation system and the degree of prolongation of the test. Because the procedure includes mixing patients with normal plasma, any individual or multiple coagulation factor deficiencies, such as those induced by warfarin, can be easily corrected.³¹

High titres of circulating anticardiolipin antibodies were also related to the presence of lupus anticoagulant.⁵⁵⁻⁵⁶ In fact, over the past few years, several reports have focused on a new clinical entity, the antiphospholipid syndrome, characterised by the presence of anticardiolipin antibodies, lupus anticoagulant, thrombosis and fetal loss.⁵⁷⁻⁵⁸ On the other hand, recent results suggest that the patient population defined by the presence of lupus anticoagulant may be distinct from, but may overlap with the anticardiolipin antibody positive population: lupus anticoagulant and antiphospholipid antibodies seem to define two distinct but related patient populations, each associated with an increased risk of thrombosis,²⁹⁻⁵⁹ while the association between anticardiolipin antibody titres and recurrent miscarriage is still debated.⁶⁰

In 36 patients with SLE we preliminarily found a strong association between a history of thrombotic episodes and the presence of lupus anticoagulant, demonstrated by the prolonged dilute aPTT. This association, however, was absent if lupus anticoagulant was screened by aPTT, which was abnormal in a lower percentage of patients with thrombosis.²⁹ This supported the suggestion that the method used so far for detecting lupus anticoagulant could have underestimated the association between lupus anticoagulant and thrombosis.

Table 6 Correlation of dilute aPTT, dRVVT, KCT, aPTT with anticardiolipin antibody tests

	Anticardiolipin antibodies	IgG	IgM	IgA
Dilute aPTT	-2.19 (0.03)	2.67 (0.01)	0.08 (NS)	0.78 (NS)
dRVVT	-2.58 (0.01)	3.21 (0.02)	1.05 (NS)	1.13 (NS)
KCT	-0.9 (NS)	1.6 (NS)	-0.6 (NS)	0.67 (NS)
aPTT	-1.8 (NS)	1.43 (NS)	-0.5 (NS)	0.62 (NS)

The table contains Z and two-tailed probability for each measure of association.

This study has shown that dilute aPTT is significantly more sensitive to thrombotic events in patients with SLE than other tests such as aPTT, KCT, and dRVVT previously shown to be diagnostic for lupus anticoagulant. In fact, among patients with SLE with past thrombotic events, the number of lupus anticoagulant positive patients was significantly higher (79%) when a dilute aPTT diagnostic test was used. Dilute aPTT also had good specificity to thrombotic events, although this was not significantly different from the other tests examined. The dRVVT showed a good sensitivity to thrombotic phenomena, even if it was less than dilute aPTT. The dRVVT, however, had better specificity, which, according to previous reports,²² was significantly higher than the anticardiolipin antibody titre. The latter, on the contrary showed poor specificity to thrombotic events; in fact, nine patients with SLE and without a history of arterial and venous thrombosis and/or miscarriages had high values of circulating anticardiolipin antibodies.

aPTT was the most specific assay to thrombotic events; this particularly high specificity could explain its low sensitivity to lupus anticoagulant.^{22 52}

Previous reports have shown that abnormal dRVVT and high anticardiolipin antibody titres were significantly associated with past presumed thrombotic events in patients with SLE.²² Our study extends this observation showing that the abnormality of dilute aPTT, dRVVT, KCT and aPTT was significantly related to thrombotic episodes, thus supporting the hypothesis of a striking association between lupus anticoagulant and thrombosis.⁶¹⁻⁶⁴ High titres of circulating antibodies, however, were not found to be significantly associated with presumed thrombotic events, except for a weak association found only in patients with arterial and venous thrombosis. Increased anticardiolipin antibody titres showed a good sensitivity to thrombotic phenomena which was not significantly different from dilute aPTT, but their low specificity probably accounted for a weak association with thrombosis. This confirms the data of Triplett, who did not find any correlation between the titre of circulating antiphospholipid antibodies and thrombotic complications in patients with the lupus anticoagulant.⁵⁹

When the statistical analysis was performed on patients with SLE and miscarriages alone, the clinical event was associated with the abnormality of dilute aPTT and dRVVT, while no association was found with the other tests examined. This suggests that in our patients with SLE the miscarriage could have been related to the presence of lupus anticoagulant. This was not found in a previous study where only aPTT, dRVVT and anticardiolipin antibodies were used to detect lupus anticoagulant.²² Perhaps an association between miscarriage and lupus anticoagulant can be found only using some laboratory tests that are very sensitive and specific to lupus anticoagulant. However, such an association is weaker than that found between arterial and

venous thrombosis and lupus anticoagulant, indicating that other mechanism(s) could be involved in the pathogenesis of miscarriage in subjects with SLE.

Finally, anticardiolipin IgG antibodies were significantly correlated with dilute aPTT and dRVVT values, confirming previous reports.^{22 29 65} This indicates that high circulating anticardiolipin IgG antibody titres do have a role in lupus anticoagulant events, although the poor specificity to thrombotic events suggests that other antiphospholipid antibodies are involved in lupus anticoagulant.⁶⁶⁻⁶⁸

In summary, among some laboratory tests usually performed to study lupus anticoagulant, dilute aPTT, a simple and cheap coagulation assay, supported by the confirmatory procedure, was the most sensitive test to past thrombotic episodes and miscarriages in patients with SLE. The combination of this assay with a very specific test like the dRVVT could help to identify patients with lupus anticoagulant who are at high risk of thrombosis.

We thank Drs T Exner and D Triplett for their helpful comments.

Supported in part by The Andrea Cesalpino Foundation.

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