Detection of lupus like anticoagulant: current laboratory practice in the United Kingdom

The Lupus Anticoagulant Working Party

Abstract

Various tests have been advocated for the detection of lupus like anticoagulants (LA) and related antiphospholipid antibodies, but there is no agreement on the most appropriate laboratory approach. Two hundred and fifty five of 433 hospital centres in the United Kingdom responded to a questionnaire. Many different tests were reported to be in use for screening for LA with considerable variation in plasma preparation, choice of reagent, and methodological details. Three freeze dried plasmas were subsequently assessed for the presence of LA by 183 laboratories. While 92% correctly identified a strong inhibitor and 91% a negative control, only 65% correctly identified a weak inhibitor. Pronounced variations in the suitability of commonly used reagents in the activated partial thromboplastin time test (APTT) were noted and important methodological features were identified in the kaolin clotting time, dilute thromboplastin time, and dilute Russell's viper venom time tests.

It is concluded that careful plasma preparation, with avoidance of platelet contamination, use of a suitable test in addition to the APTT, and attention to methodological detail are essential for the reliable identification of LA, a clinically important inhibitor.

There has been a considerable increase in interest in lupus like anticoagulants (LA) in recent years subsequent to the recognition of their association with thromboembolic and neurological disease and with spontaneous abortion.[1 2] They are a common cause of prolongation of the activated partial thromboplastin time (APTT) and occur in many different clinical situations as well as in otherwise asymptomatic subjects. LA interfere with phospholipid dependent coagulation tests, being directed against coagulation active phospholipids and inhibiting the formation of the phospholipid dependent complexes in the coagulation cascade. Several tests have been advocated as being more sensitive and specific than the APTT, but there is no consensus on the most appropriate laboratory methodology. Although a previous working party (of the International Society for Thrombosis and Haemostasis) surveyed two methods for the detection of LA and made recommendations, recent experience would suggest that some clinically important LA would remain undetected using these methods and criteria.[3] In view of the undoubted clinical importance of LA and the lack of laboratory standardisation for their detection, we considered this topic and the current report describes an assessment of current practice within the United Kingdom and makes recommendations relating to the detection of LA.

Methods

A questionnaire was distributed to the 433 hospital centres which regularly participate in the United Kingdom National External Quality Assessment Scheme (NEQAS) in blood coagulation. Information was requested on the performance of screening tests for LA, indications for testing, type of tests used, methodological detail and preanalytical variables including duration and force of centrifugation and plasma storage conditions.

Three freeze dried plasma aliquots were distributed to the 183 United Kingdom laboratories identified as regularly testing for LA. Plasma was obtained by routine plasma exchange after fully informed consent had been obtained. Clinical details of the plasma donors are listed in table 1. Participants were invited to perform their inhouse methods for assessment of the presence of LA and to interpret their findings. An APTT was requested even if not routinely performed for screening for LA, and an assessment of correction with normal plasma. Results were analysed by reagent (activated partial thromboplastin time, APTT), reagent dilution (dilute thromboplastin test, DTT and dilute Russell's viper venom time, DRVVT), and by the duration of the normal control clotting time (kaolin clotting

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Table 1 Characteristics of donors and test plasmas

Plasma	*Diagnosis*	*Manifestations*	*LA Screen**	*Anticardiolipin†*
LA/01	Antiphospholipid syndrome	Venous thrombosis recurrent abortion	Positive	Negative
LA/02	Antiphospholipid syndrome	Arterial occlusion	Strong positive	Strong positive
LA/03	Myasthenia gravis		Negative	Negative

*By DRVVT with platelet neutralisation.[4]
†By enzyme linked immunosorbent assay.

Table 2 Screening tests used by centres responding to questionnaire

APTT*	81%
APTT with aluminium hydroxide absorption and heat stability†	24%
KCT	39%
DTT‡	34%
DRVVT	21%
Other	6%

*The APTT was combined with one or more other tests in 62%.
†As described by Austen and Rhymes (1975).[5]
†As described by Exner (1978).[6]

time test, KCT). The interpretation was compared with the unanimous independent interpretations of the five organising laboratories.

Results

Two hundred and fifty five centres completed the questionnaire: 183 (72%) test for LA. Of these, at the time of the questionnaire, 187 (75%) performed the tests when specifically requested and also to investigate unexplained prolongation of the APTT. The proportion of centres performing the various types of screening tests is listed in table 2. Sixty nine (38%) performed only APTT based tests; (31%) screened by APTT alone, a negative result being taken to exclude the presence of LA. Only three laboratories used more than one APTT reagent. Reagents most commonly used were Diagen BA and Diagen KP (Diagnostic Reagents Ltd, Thame, England) 29% and 23%, respectively, Manchester regent (Manchester Thrombosis Research Foundation, Stockport, England) 29%, BCL (Boehringer Corporation Ltd, Boehringer Mannheim House, Lewes, England) 9%, GD (General Diagnostics, Organon Teknika Ltd, Cambridge, England) 7%, and others 10%.

Centrifugation conditions (and hence platelet contamination) were variable. Fifty per cent of laboratories reported use of a *g* force of less than 2000 and 50% a duration of centrifugation of less than 10 minutes. Most laboratories reported use of fresh test plasma in the APTT and KCT, but the DTT and DRVVT were performed on frozen stored plasma in up to 40% of centres.

Assays for anticardiolipin were performed inhouse at 11 (6%) of the centres; 48 (81%) have access to such assays.

Table 3 APTT results analysed by reagent used

Reagent	n	LA/01 Neat* (50:50)†	LA/02 Neat* (50:50)†	LA/03‡ Neat* (50:50)†
BCL	10	1·14 (1·08)	1·42 (1·23)	1·26 (1·13)
Diagen BA	29	1·24 (1·15)	1·70 (1·45)	1·38 (1·14)
Diagen KP	16	1·18 (1·09)	1·45 (1·35)	1·30 (1·07)
Manchester	38	1·59 (1·25)	2·20 (1·57)	1·34 (1·11)

APTT expressed as a ratio of test time* (or that of a 50:50 mix)† to control value or mid-point of normal range.
‡LA/03: The APTT was prolonged and corrected with 50% normal plasma. Prolongation not due to LA.

Table 4 Interpretation of results by method used

Test	n	% Correct interpretation LA/01	LA/02	LA/03
Aluminium hydroxide absorption-heat stability test	25	23	74	75
KCT	60	82	100	74
DTT	43	77	100	90
DRVVT with a platelet neutralisation procedure	33	92	92	86

ASSESSMENT OF PERFORMANCE OF THE SCREENING TESTS

Overall analysis

Returns were received from 133 of the 183 laboratories. Often results of several methods were reported, sometimes those for several APTT methods. One hundred and forty results of APTT were available for analysis (19 for the APTT alone), 25 for the aluminium hydroxide/heat stability test, 11 for the APTT with a platelet correction procedure, 43 for the DTT, 60 for the KCT and 33 for the DRVVT.

Seventy four of 133 laboratories gave an interpretation for LA/01 of which 48 (65%) agreed with that of the organisers. The figures for LA/02 were 92 of 133 (92%) and for LA/03, 89 of 133 (91%).

Analysis of individual methods

For the APTT four reagents were used sufficiently frequently for separate analysis (table 3). Sensitivity to LA was variable among reagents and shortening of the clotting time was noted in a 50:50 mixture with normal plasma (table 3). Correction in a normal/test mixture was considered to be shown in LA/01 by 49 (66%) of participants, nine (10%) in LA/02 and 74 (83%) in LA/03.

Other tests: The interpretation of results analysed by method is listed in table 4. DTT, DRVVT, and KCT performed well. The aluminium hydroxide absorption-heat stability test did not. In the DTT low ratios were associated with use of a low reagent dilution (table 5). Only one of six laboratories using a dilution of <1 in 50 correctly interpreted LA/01, compared with 27 of 28 using a dilution of >1 in 50.

In the KCT a normal control clotting time of less than 60 seconds was associated with an incorrect interpretation of LA/01 in five of 12 instances. None of the 12 laboratories reporting a control time of greater than 60 seconds misinterpreted LA/01.

In the DRVVT accuracy of interpretation was improved by incorporation of a platelet neutralisation procedure. The value of the test: normal ratio was increased at higher phospholipid dilution.

Table 5 Test: control ratio in DTT by reagent dilution

Dilution	LA/01	LA/02	LA/03
$<1:50$	1·3	1·6	1·2
$>1:50$, $<1/5000$	1·8	2·1	1·2
$>1:5000$	2·3	2·2	1·3

Discussion

In the face of increasing interest in the clinical associations of antiphospholipid antibodies, including those identified only by coagulation

based methods, assay procedures need to be standardised. The results of our questionnaire clearly indicate that this requirement has not yet been met in the United Kingdom. Most laboratories performing coagulation tests were invited to respond. Over one quarter of the responders do not currently screen for LA, and this is likely to be an underestimate as 41% failed to respond to the questionnaire. As it is now clear that the presence of LA is associated with significant morbidity and mortality, especially from vascular occlusive events and intrauterine death, the ability to accurately screen for the abnormality is highly desirable. It is apparent from our results that there is no current consensus on the most appropriate laboratory method for the detection of LA, on the optimal choice of reagent and technique, nor on potentially important preanalytical variables. As LA depends on antiphospholipid activity the composition of the phospholipid used in coagulation tests and the potential for pretest neutralisation of the activity by phospholipid, made available from activated platelets during sample preparation and storage, are likely to be important considerations, particularly in the detection of weak LA.

The APTT is commonly used as a screening test for LA in the United Kingdom—in some laboratories to the exclusion of other tests. Our survey indicates considerable variability in the sensitivity of commonly used reagents to LA (table 3); a reagent with high sensitivity to the inhibitor should be selected for screening purposes. It is also apparent that correction by normal plasma in a 50:50 mix in the APTT is not inconsistent with the presence of a lupus inhibitor: a weak LA, such as that of LA/01, would also not be identified using the APTT method of some laboratories. A further screening test should therefore be used. False negative results were uncommon using the KCT, DTT, or DRVVT, even in the case of the weak inhibitor LA/01 (table 4). False positive results were also uncommon in the case of the DRVVT and DTT (table 4, LA/03). Our results indicate that methodological adjustments should lead to further improvement in performance in each of these tests: avoidance of low reagent dilutions in the DTT; careful plasma preparation in the KCT with avoidance of platelet contamination; and use of appropriate phospholipid dilutions and a platelet neutralisation procedure in the DRVVT. We have shown that the aluminium hydroxide absorption-heat stability test is unreliable, and its use should not be encouraged.

A further exercise is underway in which standardised methods for the detection of LA will be compared with those currently in use by participating laboratories. It is anticipated that this will allow methodological guidelines for the detection of LA in coagulation laboratories to be prepared. In the meantime we recommend that greater consideration should be given to plasma preparation, with more efficient platelet removal by filtration or double centrifugation, that reagents sensitive to the LA are selected for use in the APTT and that the APTT alone is not considered reliable for screening. The DRVVT, KCT, or DTT are acceptable additional tests and their performance can be further improved by changes to methodological detail. Until more is known about the clinical relevance of the differing specificities of individual antiphospholipid antibodies,[7] their presence should be sought, where clinically relevant, by both coagulation based assays and immunological assays such as those using cardiolipin. By using standardised techniques it may become possible to correlate clinical changes with variation in the strength of the LA over time.

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