Guidelines

GUIDELINES ON THE INVESTIGATION AND MANAGEMENT OF THE ANTIPHOSPHOLIPID SYNDROME

In 1991, the Haemostasis and Thrombosis Task Force of the British Society for Haematology published guidelines on testing for the lupus anticoagulant (LA) (Machin et al, 1991). Since then there have been major developments in our understanding of the nature of antiphospholipid antibodies (aPLs) and improvements in our knowledge of the clinical course of the antiphospholipid syndrome. These revised guidelines have been prepared against this background. They cover both clinical and laboratory aspects.

ANTIPHOSPHOLIPID REACTIVITY

Although most early observations were on LA and anti-cardiolipin (aCL), it is now clear that so-called antiphospholipid antibodies comprise a family of antibodies reactive with epitopes on proteins which are themselves complexed with negatively charged phospholipid (Roubey, 1996). Thus, many antiphospholipid antibodies require β2-glycoprotein I (β2-GP-I), a phospholipid-binding plasma protein with weak anticoagulant activity, for binding to acidic phospholipids such as phosphatidylserine and cardiolipin (Galli et al, 1990; McNeill et al, 1990; Matsuura et al, 1990). The precise relationships among β2-GP-I, phospholipid and autoantibody are disputed. One possibility is that cryptic epitopes are exposed on β2-GP-I when it binds to phospholipid. Alternatively, binding of the glycoprotein to phospholipid may concentrate the antigenic sites and promote bivalent antibody binding. Whichever mechanism is active, it also applies when β2-GP-I interacts with other negatively charged surfaces, including the plastic of an enzyme-linked immunosorbent assay (ELISA) plate. This observation has allowed the development of new, possibly more specific, assays for antiphospholipid antibodies which use purified β2-GP-I. However, other proteins share this property of binding to phospholipid in a manner that promotes interaction with antiphospholipid antibodies. These include prothrombin, annexin V, protein C, protein S, thrombomodulin and high molecular weight kininogen. The in vitro phenomenon known as LA can be due to antibodies reactive to β2-GP-I/phospholipid or to prothrombin-phospholipid. The β2-GP-I-dependent antibodies also bind in traditional anti-cardiolipin assays as the glycoprotein is present in test serum and often in assay reagents.

Despite this improved understanding of the true nature of aPL and because the clinical utility of the newer assays is incompletely evaluated, the laboratory diagnosis of antiphospholipid syndrome still relies predominantly on coagulation-based assays for LA and solid phase assays (ELISAs) using cardiolipin.

THE ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS) may be diagnosed when arterial or venous thrombosis, or recurrent miscarriage, occurs in a subject in whom laboratory tests for antiphospholipid antibody (aCL, LA or both) are positive. Because thrombotic disease, miscarriage and transient antiphospholipid antibody positivity are all common events, persistence of the positive tests must be demonstrated and other causes and contributory factors considered. Additional clinical and laboratory features are variably present in APS, particularly thrombocytopenia and livedo reticularis. Where the condition exists against a background of chronic inflammatory disease, especially systemic lupus erythematosus (SLE), it is referred to as secondary antiphospholipid syndrome to distinguish it from the primary syndrome, in which there is no evidence for another relevant underlying disease. An international workshop recently reported on a consensus statement on classification criteria for antiphospholipid syndrome (Wilson et al, 1999). These stringent criteria are intended only for use in the context of clinical and scientific investigations of APS.

The range of disease associations with antiphospholipid antibodies is extremely broad (Table I).

Pathogenesis

The mechanisms underlying the prothrombotic state in APS have not been clarified (Greaves, 1999). Whether antibodies to β2-GP-I/phospholipid are causal is unproven. A range of pathogenetic mechanisms has been reported in subjects with APS, including protein C resistance, vascular endothelial autointimunity and activation and impaired fibrinolytic capacity. Furthermore, although thrombosis in the uteroplacental vasculature has been implicated in the pathogenesis of miscarriage, placental infarction is not a universal finding and non-thrombotic mechanisms may be involved, such as failure of implantation or autoantibody binding to the trophoblast. Because pathogenicity of the autoantibodies has not been conclusively demonstrated, it remains possible that, in at least some cases, aPLs are surrogate markers for a
Table I. Clinical associations with antiphospholipid antibodies.

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<tr>
<th>Primary antiphospholipid syndrome</th>
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<td>Venous thromboembolic disease</td>
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<td>Arterial thromboembolic disease, especially thrombotic stroke</td>
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<tr>
<td>Sterile endocarditis with embolism</td>
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<td>Recurrent pregnancy failure</td>
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| Secondary antiphospholipid syndrome in rheumatic and connective tissue disorders |
| Where thrombosis and/or recurrent miscarriage occur in association with antiphospholipid antibodies in |
| Systemic lupus erythematosus       |
| Rheumatoid arthritis              |
| Systemic sclerosis                |
| Behcet’s syndrome                 |
| Temporal arteritis                |
| Sjogren’s syndrome                |
| Psoriatic arthropathy              |
| Others                            |

| Some other associations |
| In infections, including acute self-limiting and chronic infections |
| Viral, e.g. HIV, varicella, hepatitis C |
| Bacterial, e.g. syphilis |
| Parasitic, e.g. malaria |

| In lymphoproliferative diseases |
| Malignant lymphoma |
| Paraproteinaemias |

| In drug exposure |
| Phenothiazines |
| Procainamide |
| Phenytoin |
| Quinidine |
| Hydralazine |

| Miscellaneous |
| Autoimmune thrombocytopenia |
| Autoimmune haemolytic anaemia |
| Sickle cell disease |
| Intravenous drug abuse |
| Livedo reticularis |
| Guillain–Barre syndrome |

| In the absence of underlying disease |

prothrombotic syndrome with a multifactorial, possibly autoimmune, pathogenesis. Nevertheless, their detection is frequently diagnostically and prognostically useful.

Subjects with APS present to a wide range of clinical specialists and a multidisciplinary approach to investigation and management is often appropriate. This may involve, for example, clinical haematologists, neurologists, cardiologists, rheumatologists, dermatologists and obstetricians.

Clinical presentations most commonly leading to the diagnosis of APS are venous thromboembolism, arterial occlusive events and recurrent miscarriage, but antiphospholipid antibodies may also be detected incidentally in healthy subjects (Table I). aPLs occur in relation to use of some drugs, particularly chlorpromazine, and transiently after certain infections. Persistent antiphospholipid antibody positivity may be a result of chronic infection, for example in cases of syphilis, hepatitis C and HIV infection. Familial APS has been reported but is uncommon.

Incidental aPL
In a significant proportion of subjects, the detection of aPLs is incidental in apparently healthy individuals. The thrombotic risk associated with incidental aPL positivity appears to be relatively low (Finazzi et al, 1996), although moderate to high titre aCL predicted future venous thrombosis in one epidemiological study (Ginsburg et al, 1992). A clinical assessment is indicated to exclude evidence of SLE, infection and the use of relevant medications (especially chlorpromazine).

Venous thromboembolism
Objective confirmation of thrombosis is essential as immediate and long-term management decisions may rest on the diagnosis. This is particularly important in women of childbearing age. In APS, limb deep vein thrombosis, with or without pulmonary embolism, is most common. Thrombosis in unusual sites, including the cerebral venous sinuses and intra-abdominal visceral veins, is an occasional feature. Clinical assessment should include a search for additional risk factors, present in over 50% of instances, and a detailed medical and obstetric history for supporting features of APS or SLE and related disorders. As drug-induced aPLs may not carry a thrombotic risk, the drug history is relevant in the assessment of any relationship between positive tests and clinical events.

As the diagnosis of APS may influence the duration of anticoagulant therapy because of the perceived high risk of recurrence, testing for antiphospholipid antibodies at presentation with venous thromboembolism and before the institution of anticoagulant treatment may be informative. However, this is frequently not practicable in the acute situation and anticoagulant treatment should not be delayed for the purpose of performing tests for antiphospholipid antibodies. It may not be possible to reach a conclusive diagnosis of antiphospholipid syndrome until anticoagulant treatment has been suspended. This is because demonstration of persistence of positive tests is necessary and this may not be achievable in an anticoagulated patient whose only laboratory manifestation of APS is lupus anticoagulant.

Arterial occlusive events
Stroke is most prevalent, often occurring at a young age. Any cerebral vascular territory may be affected and transient ischaemic attacks are a feature. A high rate of recurrent and multiple events is reported. Peripheral arterial occlusion, with gangrene, is less common. Although an excess incidence of aPL positivity has been found in young survivors of myocardial infarction and linked to outcome, coronary thrombosis as a presenting feature of antiphospholipid syndrome appears to be less common than stroke.

Because of the high risk of recurrent events (Finazzi et al, 1996) and the probable efficacy of anticoagulant treatment, early diagnosis is again necessary for optimal management. Clinical assessment should include a thorough evaluation for evidence of SLE and related disorders and for prior thrombotic
events and/or miscarriage. Presence of livedo reticularis (which, in young subjects with stroke, is frequently associated with aPL–Sneddon’s Syndrome) may be a feature. Cardiac valve abnormalities, including sterile endocarditis, also occur in APS, but may only be identifiable on transoesophageal echocardiography. Additional risk factors for arterial thrombosis should be sought and managed.

Pregnancy morbidity
A major feature of APS is recurrent pregnancy loss, i.e. miscarriage or fetal death in three or more consecutive pregnancies. The prevalence of antiphospholipid antibodies among women with recurrent miscarriage has been reported to be between 7% and 42%. This variability may be accounted for by the lack of standardization of laboratory protocols used to detect antiphospholipid antibodies, the inclusion of women with transiently positive test results and patient selection. Using a comprehensive methodological approach with testing for both LA and immunoglobulin (Ig)G and IgM aCL, the prevalence of persistently positive tests for aPLs is around 15% (Rai et al. 1995a). In the women with recurrent miscarriage due to APS, the prospective fetal loss rate may be as high as 90% (Rai et al. 1995b). In contrast, the prevalence of positive tests in unselected women of child-bearing age is 3% and positive tests are not sensitive predictors of poor pregnancy outcome in women with no history of pregnancy complications (Creagh et al. 1991). Many women with recurrent pregnancy loss who exhibit antiphospholipid antibodies suffer only early miscarriages (<12 weeks gestation); others have both early and late (>12 weeks gestation) or late miscarriages only. Testing for antiphospholipid antibodies is therefore applicable to all women with recurrent miscarriage, especially as effective treatment is available.

There may also be an association between aPLs and other pregnancy complications, especially placental insufficiency and early severe pre-eclampsia.

Other associations
Many of these are listed in Table I. Although thrombocytopenia is frequently present in APS, haemorrhage is uncommon. The low platelet count is usually due to an immune mechanism, analogous to that in idiopathic immune thrombocytopenia (ITP). Indeed, as in ITP, the pathogenic antibodies are directed towards epitopes on platelet membrane glycoproteins and are distinct from ‘antiphospholipid’ antibodies (Godeau et al. 1997). Furthermore, aPLs have been reported in around 30% of subjects with typical ITP.

In the rare condition of ‘catastrophic antiphospholipid syndrome’ there is multiorgan failure due to extensive microvascular thrombosis. The mortality rate is high.

INDICATIONS FOR LABORATORY TESTING FOR aPL

Because of the high risk of thrombosis recurrence and miscarriage and the potential for positive aPL tests to influence therapy, screening can be justified in a wide range of subjects.

In relation to venous thromboembolism, all subjects with apparently spontaneous events should be considered for testing. The prevalence of positive tests is likely to be lower in those with post-operative and pregnancy-related events. Recurrent venous thromboembolism, even in the presence of other risk factors, may be an indication for testing for antiphospholipid antibodies.

Subjects with stroke and those with peripheral arterial occlusive events occurring at a young age (for example less than 50 years) should be tested for aPLs, especially when risk factors for atheromatous arterial disease are not prominent. The case can be made for screening older subjects who are non-smokers and are not exhibiting other risk factors such as hypertension, diabetes mellitus or dyslipidaemia. Where recurrent arterial occlusive events occur despite antithrombotic prophylaxis, APS should be excluded.

In subjects with SLE, aPLs should be sought as part of the assessment of the autoantibody profile, as risk of thrombosis is higher in those with aPLs and the finding may influence the use of prophylactic measures at times of particular risk.

Because miscarriage is a common phenomenon, screening for aPLs is not informative after a single event. In women with three or more consecutive pregnancy losses, testing for aPLs should form part of the comprehensive investigation, including gynaecological, hormonal and chromosomal assessments. It is possible that screening for aPLs could be usefully extended to include women who do not fulfil the above strict criteria for APS but who have repeated miscarriage, defined as two miscarriages or three or more non-consecutive miscarriages. Unexplained loss of any morphologically normal fetus in the second or third trimester may be an indication for testing for aPLs. Consideration should also be given to the possible diagnosis of APS in women with early severe pre-eclampsia or severe placental insufficiency in any pregnancy. Because maternal antiphospholipid antibodies may be downregulated during pregnancy (Kwak et al. 1994), tests are best performed preconceptually or early in pregnancy when possible. A small proportion of women with aPLs also have anti-Ro antibodies. Their detection is important as it is associated with a 2% risk of fetal heart block.

LABORATORY INVESTIGATION

The diagnosis of APS relies on the demonstration of the presence of either LA by coagulation tests or aPLs by solid phase immunoassays. The latter typically use cardiolipin as the antigen (aCL assays), but some methods use phosphatidylserine. Recently, methods using purified β2-GP-I in the absence of any phospholipid have been reported, but the role of these assays in diagnosis and management has not been fully determined. There is, though, some preliminary evidence that the presence of β2-GP-I antibodies associates more strongly than aCL with thrombotic clinical events in APS (Cabides et al. 1995; McNally et al. 1995; Tsutsumi et al. 1996; Guerin et al. 1997). At present, the traditional tests remain the mainstay of laboratory investigation of APS and it is clear that both LA and solid phase-type assays must
be used for the detection of aPLs in certain patients, as cases with LA but no aCL and vice versa are well recognized. Reliance on just one type of assay may lead to false negative aPL assessments.

Coagulation assays (lupus anticoagulant tests)
In recent years, both national (Machin et al, 1991) and international (Brandt et al, 1995) guidelines for the detection of LA have been published. A large number of diagnostic tests and modifications have been described and a number of commercial kits and reagents have been introduced. Despite this, criteria for the presence of LA remain unchanged.

1. Prolongation of a phospholipid-dependent coagulation test.
2. Evidence of an inhibitor demonstrated by mixing studies.
3. Confirmation of the phospholipid-dependent nature of the inhibitor.

Optional additional criteria are the failure to demonstrate any inhibitory activity directed against a specific coagulation factor and isolation of immunoglobulin with LA activity. These remain beyond the scope of most clinical haematology departments. LA may also result in non-parallel data and spuriously low values in coagulation factor assays.

In principle, the laboratory tests should use a detection or screening stage and a confirmation stage (Table II), the latter often performed using different reagents in the same type of test. The screening stage must be as sensitive as possible to eliminate false negative results whereas the confirmation stage confers specificity to prevent false positive results, both of which may have important clinical consequences. No LA test consistently shows 100% specificity and sensitivity and, because of the heterogeneous nature of aPLs, more than one test system should be used for detection of LA. Performance of the prothrombin time and thrombin time tests is important as the results assist in the interpretation of LA tests. Also, some LAs cause interference in coagulation factor assays and should be suspected where unexpected apparent combined factor deficiencies or non-parallel dilution curves are found. Care is required to avoid misdiagnosis of coagulation factor deficiency in subjects with prolongation of the activated partial thromboplastin time (APTT) due to unsuspected LA.

Preanalytical variables
In common with the collection and preparation of blood samples for other haematostatic tests, minimal venous stasis, rapid draw and immediate anticoagulation are essential. Plasma should be prepared within 1 h of blood collection by centrifugation at room temperature at 2000 \( g \) for 15 min. Contamination with platelets and other blood cells must be minimized as these will limit the sensitivity of tests, particularly after freezing plasma samples. This may be achieved in various ways: by pipetting the plasma into a polypropylene tube and repeating the centrifugation step; by centrifugation for 5 min at 10 000 \( g \) in a microcentrifuge, which gives better platelet depletio; or by slow filtration through a 0.2 \( \mu \)m cellulose acetate syringe filter. A platelet count of less than 10 \( \times 10^9 l \) must be achieved. These considerations also apply to the preparation of control plasmas and quality control (QC) samples.

Local reference ranges must be established for each LA method and type of coagulometer. This should be accomplished by performing the LA test on at least 20 individual plasma samples from healthy normal subjects. Where ratios are used, the clotting time of each plasma is divided by the mean time, and the mean \( \pm 2SD \) range determined. Pooled normal plasma used for the calculation of LA clotting time ratios must include donations from at least 12 healthy normal subjects.

Screening tests for LA
(i) Activated partial thromboplastin time (APTT). The APTT is frequently used as the initial screening test for LA. There is great variability in the composition of APTT reagents in terms of the activator and phospholipid used. The characteristics of the phospholipid component of the APTT reagent appear to be critical in determining its LA sensitivity, and reagents vary in both the types of phospholipid present and in their relative concentrations (Kelsey et al, 1984). This variability results in inconsistent sensitivity of the test (Brandt et al, 1991). The acute phase reaction and pregnancy are associated with increased levels of fibrinogen and factor VIII, which tend to shorten the APTT and could mask a weak lupus anticoagulant. Therefore, a normal APTT is insufficient to exclude LA and additional tests must be performed.

A number of modifications of the APTT have been described for use as screening tests for LA, including the use of dilute APTT reagents (Alving et al, 1992), ratios of the APTT performed with LA-sensitive and -insensitive reagents (Brancaccio et al, 1997) and hexagonal phase phospholipids, which correct the clotting time (Rauch et al, 1989).

(ii) Dilute Russell’s viper venom time (DRVVT). In the DRVVT (Thiagarajan et al, 1986), Russell’s viper venom (RVV) activates factor X, which in turn activates prothrombin in the presence of calcium ions, factor V and

Table II. Procedures used for the detection of LA.

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<th>Screening tests</th>
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<td>KCT</td>
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<th>Confirmation of the presence of LA</th>
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<td>High-concentration phospholipid</td>
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<td>Comparison with a similar, insensitive test (e.g. Ecarin time)</td>
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phospholipid, leading to the formation of a fibrin clot. If the venom is diluted to give a suitable clotting time and the phospholipid reagent is diluted so that its concentration becomes rate limiting, any inhibition of the coagulant active phospholipid by LA results in a prolonged DRVVT.

A number of commercial DRVVT kits are now available but they vary considerably in sensitivity and specificity (Medical Devices Agency, 1998) and performance is influenced by the type of coagulometer used (Lawrie et al., 1999). It is therefore essential to use a normal reference range for each commercial kit and instrument used. Most kits and methods use some form of correction or confirmation reagent (see below).

With regard to the methodological considerations, generally the upper limit of normal for the ratio with dilute phospholipid is between 1.1 and 1.2. A method is described in the BCSH guidelines (Machin et al., 1991). If ‘in-house’ reagents are used, the RVV reagent must be titrated to give a clotting time suitable for the end-point method in use (usually 20–40 s). The correct dilution of phospholipid must then be determined to obtain optimal sensitivity and specificity of the test. If the concentration is too high, the test may be insensitive to weak LA and specificity may be lost with variations in factor levels having too great an affect on the results. The composition of the phospholipid reagent is also important and affects sensitivity. The optimal dilution can be selected by titrating the reagent with a normal plasma and a known LA-positive plasma. The dilution of phospholipid reagent that just starts to give a prolonged clotting time with normal plasma and shows a significant prolongation with LA plasma should be selected. The reagent may then be stored in aliquots at −70°C or the dilution may be noted for future tests with the same batch of reagent. New batches must be titrated and checked against the previous batch. The stability of the reagents also varies and some deteriorate significantly at 4°C during half a working day.

(iii) Other snake venoms used for LA testing. Several venoms that activate prothrombin directly have been assessed for LA testing. Taipan (Oxyuranus scutellatus) venom activates prothrombin in the presence of phospholipid and calcium ions, so that if a suitable dilute phospholipid is used the test becomes sensitive to LA (Rooney et al., 1994). The specificity can be improved by the use of mixing tests and/or a confirmation step (unpublished observations) using a platelet neutralization procedure.

Textarin (Pseudonaja textilis) venom acts in a similar fashion but also requires the presence of factor V. The specificity is again improved by mixing tests, although the performance of an additional test using a further snake venom, Ecarin, has been recommended (Tripplett et al., 1993). Ecarin is an enzyme purified from the venom of Echis carinatus, which activates prothrombin but with no requirement for phospholipid. Thus, the test is not affected by the presence of LA and the clotting time will therefore be prolonged with Textarin, but normal with Ecarin, giving a high Textarin-Ecarin ratio.

These venom tests may be useful additional methods in equivocal cases but they are not recommended as first-line tests.

(iv) Kaolin clotting time (KCT). In the KCT test, no additional phospholipid is used. The methodological considerations for this test are that the sensitivity of the kaolin clotting time (Exner et al., 1979) to LA is thought to depend on the influence of residual cell membrane fragments and plasma lipids on coagulation. For this reason, the KCT is particularly sensitive to platelet contamination of the plasma sample, which greatly reduces the sensitivity of the test, especially after freezing and thawing. LA is identified when the KCT fails to correct even after relatively large proportions of normal plasma are added, whereas in factor deficiency the KCT is corrected with small amounts of normal plasma. The use of large numbers of plasma mixtures is technically cumbersome and difficult to automate. It is therefore recommended that control and patient plasmas are tested, as well as one mixture (80% control:20% test). A test-control ratio > 1·2 indicates an abnormal result, and a mixture ratio of > 1·2 should be considered as indicative of LA whereas a ratio between 1·1 and 1·2 is considered equivocal. However, local normal reference ranges for manual methods and each automate should be established. In manual tests, a control time of < 60 s suggests contamination of the normal control plasma with platelet fragments and invalidates the result.

The KCT is not suitable for all types of coagulometer, particularly some photo-optical devices, owing to the particulate nature of the kaolin reagent, which tends to scatter light, while the stock reagent needs constant and effective stirring to prevent sedimentation. A variant of the KCT using colloidal silica (CSCT) instead of kaolin has been described previously (Chantarangkul et al., 1992) and this reagent has good optical quality, with a slow sedimentation rate, so that it is suitable for photo-optical coagulometers. The sensitivity and specificity for LA appear to be very similar to tests using kaolin. One problem common to both the KCT and CSCT is the requirement for most coagulation factors to be present in normal amounts, which means that specificity is reduced by factor deficiency or the presence of inhibitors and anticoagulants.

(v) The tissue thromboplastin inhibition test (TTI). The prothrombin time (PT) is usually normal in patients with LA and rarely shows any gross prolongation unless oral anticoagulants are used or the prothrombin concentration is low because of the presence of a specific inhibitor causing immune depletion. Thromboplastin contains very high concentrations of phospholipid, which tend to overcome the effect of LA. However, when the thromboplastin reagent is diluted, the phospholipid concentration becomes a rate-limiting factor, and any inhibition of the prothrombinase reaction by LA causes a prolongation of the modified PT. This principle has been utilized in the tissue thromboplastin inhibition test (TTI, Schleider et al., 1976).

Methodological considerations for this test are that the prothrombin time is performed using two or more thromboplastin dilutions and the ratio of test to control PT with the diluted thromboplastin is calculated. A progressive increase in the ratio with thromboplastin dilution is
suggestive of LA. The concentration and characteristics of the phospholipid component of thromboplastin reagents show considerable heterogeneity. Thus, the sensitivity and specificity range varies between reagents and batches. Although the test was not recommended in a previous guideline (Machin et al, 1991), it has recently been demonstrated that a recombinant human tissue factor mixed with synthetic phospholipids (Innovin, Dade) is particularly sensitive to LA (Arnout et al, 1994; Forastiero et al, 1994).

**Confirmatory tests**

(i) Demonstration of inhibitory activity. The presence of an inhibitor can be confirmed by the performance of mixing tests with normal plasma. In factor deficiency, the clotting time is corrected by the addition of small volumes of normal plasma, but in the presence of LA relatively large volumes of normal plasma must often be added to correct the clotting time. The addition of normal plasma may even prolong the clotting time, a phenomenon known as the lupus cofactor effect. A 50% mix with normal plasma is generally used. However, mixing tests unavoidably result in dilution of the LA antibody and this can lead to false negative results. Nevertheless, the approach is valuable as, with moderate and strong LA, the specificity of LA tests is improved.

(ii) Demonstration of phospholipid dependence. The platelet neutralization procedure (PNP) is commonly used. Washed normal platelets are activated with calcium ionophore or lysed by repeated freezing and thawing (which exposes procoagulant phospholipid). These platelets are added to plasma and their effect is compared with a buffer control. Platelets appear to bypass LA (although the mechanism of the effect is not completely understood) and will correct the clotting time, whereas in factor deficiency the clotting time remains prolonged. This principle has been used for confirmation of LA in the APTT, the DRVVT and the Taipan venom time. Several reagents for the performance of the PNP are commercially available.

A neutralization test based on platelet-derived microparticles has also been described (Arnout et al, 1992) and may be useful. An alternative method for the confirmation of LA uses a modified APTT reagent containing hexagonal phase lipids, which specifically bind LA and remove its effect on the APTT reagent.

In the DRVVT, an acceptable alternative to the PNP is the use of a ‘confirmation’ reagent which contains a high concentration of phospholipid or an LA-insensitive phospholipid: several reagents are available commercially. Confirmation reagents can alter the clotting time of LA-negative plasmas and it is therefore advisable to calculate DRVVT ratios (patient time divided by control time) for each reagent, rather than attempt interpretation of the individual clotting times.

If ‘in-house’ reagents are used in these confirmatory procedures, it is essential to carry out titration experiments similar to those described above in relation to the RVV and phospholipid reagents.

Several methods for the calculation of correction in the DRVVT have been reported and there is no consensus on the optimal approach:

(a) Percentage correction of ratio. The DRVVT ratio of test-control plasma for the dilute phospholipid reagent (DPL ratio) and for the correction or confirm reagent (CORR ratio) are calculated. Percentage correction is equal to \((\text{DPL ratio} - \text{CORR ratio}) \times 100/\text{DPL ratio}\). A result above the normal range (e.g. \(>1.1\)) with dilute phospholipid, which corrects to within the normal range, or by \(>10\%\) with the high phospholipid reagent or platelet neutralization procedure is considered indicative of LA.

(b) Percentage correction of clotting time. The control DRVVT clotting time is subtracted from the test DRVVT and the product is divided by the control DRVVT to yield a weighted ratio for dilute phospholipid (DPL) and for the correction reagent (CORR). The percentage correction is then calculated as \(100 \times ((\text{DPL} - \text{CORR}) / \text{DPL})\). In general, corrections of \(>65\%\) are indicative of LA.

(c) Test-confirm ratio. The DRVVT clotting time with dilute phospholipid is divided by the DRVVT with the correction reagent. A test-confirm ratio \(>2\) above the mean normal ratio indicates the presence of LA.

There is little objective information to support any exact percentage correction as indicative of LA, and the above limits are largely arbitrary. Correction back to within the normal range is strong evidence for the presence of LA. The use of the test-confirm ratio does not take into account any variation in the normal clotting time with the reagent, differences in operator performance or changes in reagent stability, and cannot therefore be recommended for general use. The percentage correction of ratio and the percentage correction of clotting time give the most reliable results, although the latter is slightly more cumbersome (Medical Devices Agency, 1999).

Tests for LA in subjects treated with oral anticoagulants. Accurate detection of LA may not be possible in a subject treated with warfarin. One approach is to perform the DRVVT on equal volume mixtures of normal and test plasmas. In this case, the confirm step should also be performed on a similar plasma mixture to avoid confusion because of the dilution of LA immunoglobulins. A clear positive result may be clinically useful but an equivocal or negative result is unreliable.

Taipan and Textarin venom times may be particularly useful diagnostically in plasma from patients receiving oral anticoagulants as they are relatively insensitive to prothrombin deficiency (Triplett et al, 1993; Rooney et al, 1994). As no other vitamin K-dependent factor is required, in the absence of LA, the clotting time in oral anticoagulant patients remains normal. However, unless a mixing test or confirmation procedure is used, their specificity is limited.

It is difficult, and rarely necessary, to test for LA on plasma from a subject receiving therapeutic doses of heparin, although some commercial DRVVT reagents include compounds which neutralize heparin.

Quality control. The inclusion of an internal quality control (QC) system is important in all LA tests, regardless of the instrumentation used and the source of reagents. A minimum requirement is the inclusion of known LA-positive and LA-negative plasmas in every batch of tests. Samples collected locally and stored in aliquots at \(-70°C\)
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may be used. Lyophilized commercial LA-positive plasmas are available, but many are too potent and give little information about LA sensitivity. Commercial normal plasmas may contain platelet fragments, which limits their usefulness. Positive and negative reference plasmas have been prepared in collaboration between the British Committee for Standards in Haematology and the National Institute for Biological Standards and Control (‘First British Reference Plasma Panel for LA 96/522, 96/560, 96/544’, available from NIBSC, South Mimms, Potter’s Bar, UK).

The UK National External Quality Assurance Service (NEQAS) facilitates external quality assessment of LA tests by the distribution of plasma samples to all participating centres. Recent exercises have highlighted ongoing problems in the accurate identification of LA (Jennings et al., 1997).

Solid phase assays (for aCL and β2-GP-I antibodies)

Solid phase assays for aPL, such as the aCL ELISA test, have been gradually refined (Loizou et al. 1987; Khamashita & Hughes, 1993). The ELISA format allows bulk testing and the results are not affected by factor deficiency or the use of anticoagulants.

Important methodological considerations are the quality of the cardiolipin used and the technique for coating the microtitre plates. The binding of most aCL requires the presence of the protein cofactor β2-GP-I, which is essential for satisfactory and reproducible aCL results. This is provided by fetal calf serum or adult bovine serum in the blocking agent (used to reduce non-specific binding to the microplate) and/or sample diluent. The use of uncoated wells as sample blanks is essential to reduce the effect of non-specific binding due to interfering substances in the test sample (e.g. rheumatoid factor). ELISA assays based on other single phospholipids have been described previously (e.g. phosphatidylserine), but these appear to have similar specificity to aCL assays and similar limitations.

It is recommended that the aCL test is standardized by the use of affinity-purified standards (Harris et al. 1987) or secondary standards derived from these. These allow the calculation of aCL results in IgG or IgM antiphospholipid units (GPLU and MPLU respectively) related to a given concentration of affinity-purified aCL immunoglobulin.

Positive and negative QC sera should also be included in each batch of assays. An external quality control service is available in the UK from NEQAS Immunology.

The detection of aCL allows the diagnosis of APS in a subject with an appropriate clinical history, even when LA is absent. However, the aCL assay is not a substitute for the LA test nor does it confirm that LA is present because different antibodies appear to be responsible for the two activities. Furthermore, the clinical significance of low-titre aCL is doubtful. Thus, in cases where the aCL titre is less than 20 GPL units and tests for LA are negative, a diagnosis of APS may not be conclusive. Under these circumstances, it is particularly important to consider other causes of thrombosis or miscarriage. There is also debate over the importance of the aCL isotype. IgG antibodies may be more clinically significant, although IgM aCL appears to be associated with thrombotic events and miscarriage in some series. Tests for IgA antibodies may not be clinically informative (Selva-O’Callaghan et al. 1998) and their use is not currently recommended.

A variety of specific assays for β2-GP-I antibodies have been developed (Roubey et al. 1996) and several commercial kits are available. β2-GP-I antibody assays show higher precision and better correlation with the thromboembolic complications in APS and SLE than assays for aCL and are less likely to show transient positive results in association with infection. The problems of standardization remain, however. Gamma-irradiated (high antigen binding) polystyrene plates or other plates with a high density of surface electrostatic charge must be used to ensure efficient β2-GP-I binding. Also, β2-GP-I preparations which are impure, or have undergone proteolytic cleavage, should not be used.

Occasionally, sera are aCL negative but are β2-GP-I antibody positive. This is usually due to species-specific antibodies which fail to bind to bovine β2-GP-I in aCL assays. These antibodies are nevertheless clinically significant because they may be associated with APS. aCL-positive β2-GP-I antibody-negative samples may contain other autoantibodies, including those reactive with annexin V, protein C, protein S or prothrombin. These may be provided by the blocking agent, sample diluent or by the test sample itself. Antiprothrombin antibodies generally exhibit poor specificity for venous thrombosis and recurrent fetal loss and may be found in patients with infection. Their precise clinical significance is not yet clear. One report has claimed an association with myocardial infarction (Vaarala et al. 1996) but more work is required to clarify the clinical importance of this observation.

SUMMARY OF THE RECOMMENDED APPROACH FOR THE DETECTION OF LA (FIG 1)

Attention must be paid to preanalytical variables.

A coagulation screen should be performed to identify unexpected factor deficiency or anticoagulant effect.

The APTT may be used as a screening test for LA. A sensitive reagent must be used.

If the APTT is prolonged, performance of mixing tests with normal plasma and/or a PNP is frequently informative.

A second test should also be used, preferably the KCT or the DRVVT with a correction procedure.

When results in these tests are equivocal, supplementary tests using different methods may be of help, e.g. other venom tests or the TTI.

Immunosassay for IgG and IgM aCL (and/or possibly for β2-GP-I antibodies) must also be performed.

The presence of aPL must be confirmed in separate blood samples collected at least 6 weeks apart to demonstrate persistent positivity. (It should be noted, however, that the finding of a single positive test for aCL in subjects with deep venous thrombosis was predictive of an increased risk of recurrent thrombosis in the study of Schulman et al. (1998), even in those in whom subsequent tests were negative.)

In subjects receiving oral anticoagulants, accurate
identification of LA may not be possible. The KCT and DRVVT performed on mixtures of control and patient plasmas, or the Taipan and Textarin times, may be useful.

MANAGEMENT

Useful data are beginning to emerge that guide optimal management of thrombosis and miscarriage in APS.

Incidental finding of aPL
Prospective data generally indicate a low risk of thrombosis in this group, although some reports suggest an increased level of risk over background (Ginsburg et al., 1992). Antithrombotic therapy is therefore not usually indicated when there has been no thrombotic event (level III evidence, grade B recommendation). As it is likely that this group includes subjects with APS who have not yet had a first event, a low threshold for use of thromboprophylaxis at times of high risk is indicated. Use of short-term heparin prophylaxis perioperatively is prudent. Longer-term anticoagulant exposure, for example in pregnancy and after childbirth, in the asymptomatic woman with positive tests is not justified at present as the associated risk of iatrogenic morbidity may outweigh that of thrombosis. It is not known whether the risk of thrombosis associated with use of the combined oral contraceptive or hormone replacement therapy (HRT) is further enhanced in the presence of aPL in an asymptomatic woman. In the absence of prospective data, decisions regarding the use of hormonal contraception and HRT in women with incidental aPL can only be made on pragmatic grounds, taking into account other considerations such as the acceptability and safety of alternative approaches and the presence of additional thrombotic risk factors.

Venous thromboembolism in APS
The initial management of the acute event, with intravenous monitored unfractionated or subcutaneous low molecular weight heparin is not influenced by the diagnosis of APS (level III evidence, grade B recommendation). Warfarin therapy should be instituted in the usual way, with a target international normalized ratio (INR) of 2.5 (optimal range 2.0–3.0). There is controversy over the requirement for more intensive anticoagulant therapy, the duration of anticoagulation and the method of INR determination. Three retrospective studies suggest an ongoing risk of thrombosis at INR values of less than 3.0, and a target of 3.5 (3.0–4.0) has been recommended (Khamashta et al., 1995). There are no prospective data.

Intensity and duration of treatment should be determined on an individual basis, taking into account the presence of additional remediable risk factors, the severity of the presenting event and the particular risk of bleeding on warfarin (level IV evidence, grade C recommendation). It should be borne in mind that fewer than 1% of episodes of venous thromboembolism are fatal. Also, although significant morbidity from post-phlebitic syndrome develops in around 30% of individuals with lower limb deep vein thrombosis, a significant proportion of these eventually become asymptomatic (Prandoni et al., 1996, 1999). Conversely, the rate of life-threatening bleeding in subjects taking warfarin, based on a prospective study, is at least 0.25% per annum (Palareti et al., 1996) and this rises rapidly when the INR exceeds 4.0. Bleeding is more common in the elderly. Despite these concerns over bleeding

risk, there is increasing evidence that long-term therapy with warfarin may be advantageous in some subjects with venous thromboembolism and also that the thrombosis recurrence rates may be particularly high in APS (Schulman et al., 1998; Kearon et al., 1999). The risk-benefit ratio of long-term anticoagulation has not yet been assessed. However, therefore, for many subjects with deep vein thrombosis in antiphospholipid syndrome, treatment for up to 6 months at target INR of 2·5 and management of additional reversible risk factors is reasonable. Recurrent venous thrombosis should be treated by long-term oral anticoagulation. Recurrence while the INR is between 2·0 and 3·0 should lead to more intensive warfarin therapy, target INR 3·5 (optimal range 3·0–4·0), but this is uncommon. Use of the combined oral contraceptive and of HRT are best avoided in women with aPL and thrombosis, at least in those not on warfarin therapy, but clearly pregnancy carries its own risk of thrombotic complications. Although progestogen-only preparations have been used in women at risk of venous thrombosis, whether they are safer is not yet clear. Use of hormonal methods to ensure effective contraception while on treatment with warfarin should be considered (all level IV evidence, grade C recommendations).

Arterial thrombosis
Because of the high risk of recurrence and likelihood of consequent permanent disability or death, stroke due to cerebral infarction in APS should be treated with long-term oral anticoagulant therapy, target INR 2·5 (optimal range 2·0–3·0) (level III evidence, grade B recommendation). Higher-intensity anticoagulation has been recommended and may be appropriate in some cases (The Haemostasis and Thrombosis Task Force, 1998), but results of prospective studies are required before the use of a target INR of 3·5 can be unequivocally supported. Recurrence while on standard intensity therapy dictates the use of a higher target INR (3·5), however. Whether additional therapy with aspirin is efficacious in this situation is not known, but the risk of haemorrhage is increased when aspirin is used alongside oral anticoagulant therapy (Meade & Miller, 1995). Extracerebral arterial thromboembolic manifestations of APS will also warrant consideration of continuation of long-term anticoagulation with warfarin in many instances.

As in the case of venous thrombosis, attention should be paid to the correction and avoidance of additional thrombotic risk factors.

Anticoagulant monitoring in APS
The use of the APTT to monitor unfractionated heparin may be problematic because of prolongation of the baseline value due to LA. Use of an anti-Xa assay circumvents this problem. Treatment with low molecular weight heparin, avoiding the need for coagulation monitoring in most cases, is also an option.

It has been suggested that the INR may be misleading in subjects with LA on warfarin as the level of anticoagulation may be underestimated because of an effect of the LA on the prothrombin time (Della Valle et al., 1996; Moll & Ortel, 1997). Although this phenomenon appears to be more likely when certain recombinant thromboplastin reagents are used in the determination of prothrombin time, other reagents have also been implicated. The clinical importance of this phenomenon is not yet known, but current evidence suggests that the INR is reliable when thromboplastin with a low international sensitivity index (ISI) and calibrated for the method and equipment utilized is used in prothrombin time determination (Lawrie et al., 1998).

Thrombocytopenia
When thrombocytopenia is the only manifestation, and especially if the clinical picture is one of a haemorrhagic state, the management should be identical to that for idiopathic thrombocytopenic purpura (ITP). Splenectomy has been safely and successfully performed and is appropriate treatment if clinically indicated (Leuzi et al., 1997). Although the degree of thrombocytopenia is generally modest in APS, platelet counts of < 50 × 10⁹/l are occasionally encountered. Thromboprophylaxis with warfarin may carry an increased haemorrhagic risk in this situation, but should be considered where thrombosis is the principal clinical manifestation.

Pregnancy failure
In women with APS and a history of pregnancy complications, there is a particular need for close collaboration between specialists. A variety of treatments including corticosteroids, low-dose aspirin, heparin and immunoglobulins have been used either as single agents or in combination in an attempt to improve the rate of live births in women with antiphospholipid antibodies (Carreras et al., 1988; Silver et al., 1993; Kutteh, 1996). Available data are limited by the small number of patients in individual studies, which have also had varying entry criteria and treatment protocols, and by the lack of standardization of laboratory assays used to detect antiphospholipid antibodies. The use of corticosteroids in pregnancy is associated with significant maternal and fetal morbidity (Cowchock et al., 1992; Laskin et al., 1997), appears to be ineffective and should be avoided (level IIa evidence, grade B recommendation).

Based on the results of the only randomized controlled clinical trial reported to date (Rai et al., 1997), treatment with low-dose aspirin and heparin is applicable to women with a history of recurrent miscarriage associated with persistent antiphospholipid antibodies (level IIa evidence, grade B recommendation). Aspirin, 75 mg/d, should be commenced as soon as the urine pregnancy test becomes positive. Because the majority of miscarriages occur before 14 weeks of gestation, and thus delay in commencement of treatment may compromise the chances of a successful outcome, low-dose heparin, by self-administered subcutaneous injection, should be commenced when fetal heart activity is seen on ultrasonography. In the study of Rai et al. (1997), unfractionated heparin (5000 IU) was administered twice daily subcutaneously. Use of low molecular weight heparin preparations in once-daily subcutaneous doses is becoming increasingly popular for thromboprophylaxis, but...
whether the altered pharmacokinetics in late pregnancy allow effective once-daily use is not yet clear. At present, no low molecular weight heparin preparation is licensed for use in pregnancy, but observational studies suggest low molecular weight heparin preparations are safe alternatives to unfractionated heparin (Sanson et al. 1999). The ideal duration of heparin therapy has not been determined, but should not be unnecessarily extended because of the potential risk of heparin-induced osteopenia. Discontinuation at 34 weeks gestation is a reasonable compromise in women with no history of thrombosis in whom early pregnancy loss has been a feature. When late pregnancy complications have occurred previously, continuation of antithrombotic therapy to delivery is reasonable and postpartum thromboprophylaxis will usually also be indicated in those women with a history of thrombosis. Delivery by Caesarean section carries an additional thrombotic risk and perioperative thromboprophylaxis is indicated.

After commencement of heparin, the platelet count should be monitored. A weekly platelet count for the first 3 weeks and every 4–6 weeks thereafter is generally practicable. Regular obstetric assessment, including Doppler ultrasound fetal scanning, allows early detection of complications.

In women who do not fulfil the criteria for APS but who have persistent aPL and a previous failed or complicated pregnancy, the efficacy of treatment with aspirin and heparin in subsequent pregnancies has not been tested. Although there is often pressure to offer treatment, the potential for side-effects must be especially carefully considered in view of the unknown benefit of pharmacological intervention at this stage.

Although use of low-dose heparin and aspirin appears to improve pregnancy outcome in APS, treated pregnancies are frequently complicated by fetal growth retardation, gestational hypertension and premature delivery (Backos et al. 1997), high-

CONCLUSION

Our understanding of the pathogenesis and clinical features of autoimmune thrombotic disease is increasing rapidly. APS is a multifaceted and complex condition which is clinically demanding with respect to both diagnosis and management. There is a clear need for further studies into the pathogenetic mechanisms involved, for the development of more specific laboratory techniques to identify those patients at particular risk of thrombosis and miscarriage and for the enrolment of patients in prospective controlled trials of treatment.

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