Guidelines on the investigation and management of antiphospholipid syndrome

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Introduction

This guidance updates and replaces the previous guideline on the investigation and management of antiphospholipid syndrome (APS) published in 2000 (Greaves *et al*, 2000), though where there have not been changes we refer back to them when appropriate. The guidance is updated with reference to relevant publications since 2000. Publications known to the writing group were supplemented with additional papers identified by searching PubMed for publications in the last 11 years using the key words: lupus anticoagulant, anticardiolipin, antiphospholipid, β2–glycoprotein I, antiprothrombin and limits (clinical trial, randomized control trial, meta-analysis, humans, core clinical journals, English language). The writing group produced the draft guideline, which was subsequently revised by consensus by members of the Haemostasis and Thrombosis Task Force of the British Committee for Standards in Haematology. The guideline was then reviewed by a sounding board of approximately 50 UK haematologists, the Royal College of Obstetricians and Gynaecologists (RCOG), and the British Committee for Standards in Haematology (BCSH) Committee and comments incorporated where appropriate. The 'GRADE' system was used to quote levels and grades of evidence, details of which can be found at http://www.bcshguidelines.com/BCSH_PROCESS/EVIDENCE_LEVELS_AND_GRADES_OF_RECOMMENDATION/43_GRADE.html. The objective of this guideline is to provide healthcare professionals with clear guidance on

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the diagnosis and management of patients with antiphospholipid syndrome though individual patient circumstances may dictate an alternative approach.

Features of the antiphospholipid syndrome (APS)

The antiphospholipid syndrome (APS) is an acquired autoimmune condition. The clinical features are thrombosis (venous, arterial and microvascular) and/or pregnancy complications and failure. It is important to recognize the syndrome in the context of these problems and to institute appropriate therapy to reduce the risk of recurrence. The reader is directed to reviews published since our previous guideline (Lim *et al*, 2006; Robertson & Greaves, 2006; Ruiz-Irastorza *et al*, 2007; Giannakopoulos & Krilis, 2009; Giannakopoulos *et al*, 2009).

Definitions

Antiphospholipid syndrome is diagnosed in a patient with thrombosis and/or defined pregnancy morbidity (see below) who has persistent antiphospholipid antibodies (aPL). Venous thrombosis in APS is most commonly lower limb deep vein thrombosis (DVT) and/or pulmonary embolism (PE) but any part of the venous system may be involved, including superficial, portal, renal, mesenteric and intracranial veins. The most frequent site of arterial thrombosis in APS is in the cerebral vasculature resulting in transient cerebral ischaemia/stroke. Myocardial infarction is less common, although subclinical myocardial ischaemia may be under-recognized (Sacre *et al*, 2010). Despite these clear associations between aPL and thrombosis, APS makes only a minor contribution to the overall burden of disease from VTE and stroke. Microvascular thrombosis in APS is least common but may manifest as the potentially lethal 'catastrophic antiphospholipid syndrome' (CAPS). In CAPS there is typically multiorgan failure involving, but not confined to, the lungs, brain and kidneys.

Historically aPL have been detected as either a lupus anticoagulant (LA) or as anticardiolipin antibodies (aCL). LA is an *in vitro* phenomenon in which there is prolongation of a phospholipid-dependent coagulation test that is not due to an inhibitor specific to a coagulation factor (see Section 'Lupus anticoagulant testing'). It was originally thought that the LA phenomenon was due to autoantibodies against anionic phospholipids interfering with the assembly of the tenase and prothrombinase complexes, and the aCL assay (see Section 'Solid phase aPL assays') was developed as an alternative way of detecting these hypothetical antibodies. However it became clear in the early 1990s that these tests were detecting antibodies not to anionic phospholipids but to phospholipid binding proteins. The aCL enzyme-linked immunosorbent assay (ELISA) typically detects antibodies to β_2–glycoprotein I (β_2GPI) (Galli *et al*, 1990; McNeil *et al*, 1990) and LA tests are sensitive to antibodies to β_2GPI (anti-β_2GPI) and also antibodies to prothrombin (Bevers *et al*, 1991).

β_2GPI is an apolipoprotein and a member of the complement control protein family; it binds to cell surface receptors and negatively charged surfaces. Among anti-β_2GPI it has been demonstrated that it is those that bind specifically to a limited epitope on domain 1 of the protein (Gly40-Arg43) that are most strongly associated with thrombosis (de Laat *et al*, 2005). Antiprothrombin antibodies are weakly associated with thrombosis; they usually have a low affinity, but in some patients higher affinity antibodies are produced which cause the rare complication of hypoprothrombinaemia.

APS has been described as secondary if there is an associated autoimmune disorder, such as systemic lupus erythematosus (SLE) or rheumatoid arthritis, and primary if not. In order to ensure consistency in research, consensus criteria for the diagnosis of APS have been agreed (Miyakis *et al*, 2006) (Table I).

Whilst these criteria are useful for encouraging uniformity in clinical studies their uncritical application to the individual case in the clinic should be avoided; rather, the diagnosis should depend upon a thorough assessment of the clinical history, consideration of alternative causes of thrombosis or pregnancy morbidity and review of the laboratory data in the light of knowledge of the limitations of the assays (see Section 'Detection of aPL in the clinical laboratory').

Clinical associations

In addition to thrombosis and pregnancy morbidity there have been many claims of other clinical associations with aPL. Thrombocytopenia, heart valve disease (which is most commonly occult), chorea, livedo reticularis/racemosa and nephropathy are likely associations, although like the thrombotic and pregnancy manifestations, none is specific to APS (Miyakis *et al*, 2006). Transverse myelopathy occurs in SLE and may be more frequent in those with aPL (Cervera *et al*, 2002). A purported association with infertility has not been substantiated (Buckingham & Chamley, 2009) and an association with migraine is controversial with one recent study finding a relationship (Cavestro *et al*, 2011) but others not (Montalban *et al*, 1992; Tietjen *et al*, 1998). Another controversial concept is that APS may manifest as a disorder closely mimicking multiple sclerosis and responsive to anticoagulant therapy (Hughes, 2003). However, aPL may be present in some cases of otherwise typical multiple sclerosis (Heinzlef *et al*, 2002) perhaps representing an epiphenomenon in a disorder with an immune pathogenesis. Even more controversial is the suggestion that there may be a seronegative form of APS (Hughes & Khamashta, 2003). The principal manifestations of APS, thrombosis and pregnancy failure, are common and in most cases have no autoimmune basis; as such the diagnosis of 'seronegative APS' would be difficult to

Table I. Research criteria for defining the antiphospholipid syndrome. Adapted from Miyakis *et al* (2006). With permission, John Wiley & Sons, Inc. © 2006 International Society on Thrombosis and Haemostasis.

Clinical criteria
  1. Vascular thrombosis
      One or more clinical episodes of arterial, venous or small vessel thrombosis
  2. Pregnancy morbidity
      (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
      (b) One or more pre-term births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia or (ii) recognized features of placental insufficiency
      (c) Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory criteria
  1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart
  2. Anticardiolipin (aCL) antibody of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. >40GPL units or MPL units, or > the 99th centile), on two or more occasions, at least 12 weeks apart
  3. Anti-β2–glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titre >the 99th centile), present on two or more occasions at least 12 weeks apart
Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria are met

GPL units, IgG antiphospholipid units; MPL units, IgM antiphospholipid units.

sustain. This guideline considers only thrombosis (primarily venous thromboembolism and arterial ischaemic stroke) and pregnancy morbidity, in APS.

aPL and thrombosis. In relation to venous thrombosis Galli *et al* (2003a,b) published two papers, which looked at the evidence for an association with aPL. There was evidence of an association with LA, odds ratios (OR) across studies ranging from 4·1 to 16·2. Although some studies suggested an association with aCL (Ginsburg *et al*, 1992; Schulman *et al*, 1998), others did not (Stegnar *et al*, 1991; Bongard *et al*, 1992; Oger *et al*, 1997) and overall Galli *et al.* concluded that aCL were not independently associated with DVT. For anti-β_2GPI the same authors found 7/14 studies showed a significant association with venous thrombosis but only in retrospective studies. In 2004 β_2GPI dependent LA was shown to be associated with venous thrombosis (de Laat *et al*, 2004). The following year the presence of IgG anti-β_2GPI was shown to predict thrombosis in patients with LA (Zoghlami-Rintelen *et al*, 2005). An analysis of the Leiden Thrombophilia Study demonstrated that the presence of LA, anti-β_2GPI and anti-prothrombin antibodies are risk factors for DVT in a general population, the strongest association being for the combination of LA, aβ_2GPI and anti-prothrombin antibodies (de Groot *et al*, 2005). In a prospective population-based nested cohort study, aCL did not predict a first episode of venous thrombosis (Naess *et al*, 2006). In the WAPS study (Galli *et al*, 2007) IgG anti-β_2GPI were associated with thrombosis whereas IgM anti-β_2GPI, IgG aCL and IgM aCL were not. The authors proposed that anti-β_2GPI replace aCL measurement and that only the IgG isotype should be tested for.

With regard to arterial thrombosis the aforementioned reviews found that both LA and IgG aCL were associated with arterial thrombosis but that IgM aCL were not (Galli *et al*, 2003a,b). For anti-β_2GPI they found 3/10 studies showed a significant association with arterial thrombosis and concluded that the evidence did not support an association with arterial events. β_2GPI-dependent LA has been shown to be associated with arterial thrombosis (de Laat *et al*, 2004). In the RATIO (Risk of Arterial Thrombosis in Relation to Oral. Contraceptives) study of 175 patients with ischaemic stroke and 203 patients with myocardial infarction (Urbanus *et al*, 2009) the OR of LA for myocardial infarction was 5·3 (95% confidence interval [CI] 1·4–20·8) and for ischaemic stroke 43·1 (12·2–152·0). In women who had anti-β_2GPI antibodies the risk of ischaemic stroke was 2·3 (1·4–3·7), but the risk of myocardial infarction was not increased (0·9, 0·5–1·6). Neither aCL nor antiprothrombin antibodies affected the risk of myocardial infarction or ischaemic stroke.

There are fewer data on antibodies of IgA isotype but inclusion of IgA aCL tests does not improve diagnostic efficiency (Bertolaccini *et al*, 2001; Samarkos *et al*, 2006).

In general, among aPL, the specificity for thrombosis is higher for LA than aCL or anti-β_2GPI and greater for higher than lower titre aCL. In one study, patients positive for a LA in both a dilute Russell viper venom time (DRVVT) and a sensitive activated partial thromboplastin time (APTT) were more likely to have thrombosis than patients with only one positive LA test (Swadzba *et al*, 2011). IgM and IgA antibodies are poorly specific. In addition, among patients with thrombosis, the highest risk of recurrence is the relatively small cohort positive for all of LA, aCL and anti-β_2GPI (Pengo *et al*, 2010).

aPL and pregnancy morbidity. There is substantial evidence linking aPL to an increased risk of recurrent and late pregnancy loss (Ginsberg *et al*, 1992; Rai *et al*, 1995; Laskin *et al*, 1997; Robertson *et al*, 2006). LA has a stronger association with pregnancy loss than the other anti-phospholipid antibodies, while the importance of anti-β_2GPI and pregnancy loss is uncertain (Opatrny *et al*, 2006). In the meta-analysis by Opatrny *et al* (2006), both IgG and IgM aCL were associated with recurrent fetal loss but it was not possible to determine the significance of isolated IgM aCL as studies have not distinguished between women having isolated IgM aCL and women having additional aPL antibodies.

With regard to pre-eclampsia, placental abruption and fetal growth restriction (FGR), there is an association between these complications and the presence of aPL but this is less strong than with recurrent pregnancy loss (Branch *et al*, 2001; Robertson *et al*, 2006).

Pathophysiology

Whether the association of aPL with thrombosis is causal has been contentious though studies in experimental animals do suggest that aPL are directly prothrombotic (Blank *et al*, 1991). Many mechanisms for thrombosis in APS have been suggested, such as increased expression of tissue factor on monocytes and endothelial cells (Branch & Rodgers, 1993; Amengual *et al*, 1998), interference in the protein C anticoagulant pathway (Malia *et al*, 1990; Atsumi *et al*, 1998a), inhibition of fibrinolysis (Atsumi *et al*, 1998b) and inhibition of annexin V binding to phospholipids (Rand *et al*, 1998). More recently attention has focused on anti-β_2GPI (see Giannakopoulos *et al* (2007) for a review). β_2GPI can exist in two conformations in plasma (Agar *et al*, 2010), a closed circular form and an open form. The circular conformation is maintained by interaction between the first and fifth domain of β_2GPI, in the open conformation a cryptic epitope in the first domain becomes exposed, enabling antibody binding. Antibody-β_2GPI complexes bind to a variety of receptors (e.g. Toll-like receptors 2 and 4, annexin A2, glycoprotein 1bα, and LRP8 in the LDL receptor family) on different cell types, including endothelial cells, platelets, monocytes and trophoblasts (de Groot & Meijers, 2011) and may trigger intracellular signalling and inflammatory responses.

Pregnancy failure may be due to thrombosis in the placental bed, although alternative pathogenic mechanisms may apply, and may explain the tendency to very early losses

prior to placentation. aPL appear to have a direct effect on trophoblasts, (Chamley *et al*, 1998; Nelson & Greer, 2008; Simioni, *et al* 1999) and there is evidence for activation of complement in pregnancy failure in experimental APS (Girardi *et al*, 2004; Salmon & Girardi, 2004) and in humans (Shamonki *et al*, 2007; Oku *et al*, 2009). These observations may explain the apparent efficacy of heparin in the prevention of early fetal losses in APS as heparin has been shown to exert potentially beneficial effects on trophoblasts *in vitro* (Simioni, *et al* 1999) and to inhibit complement activation in experimental APS (Girardi *et al*, 2004; Salmon & Girardi, 2004).

Detection of aPL in the clinical laboratory

The methodology for LA and solid phase aPL assays (e.g. aCL) was covered in detail in the previous BCSH guideline (Greaves *et al*, 2000).

Preparation of plasma samples

Blood should be collected into 0·109 mol/l trisodium citrate. Platelet contamination should be avoided by double centrifugation at 2000 *g* for 15 min at 15–22°C. This should yield plasma with a platelet count of <10 × 10^9/l. Plasma filtration through 0·2 μm filters is not recommended as this may generate microparticles (Favaloro, 2007). Ultracentrifugation (>5000 *g*) as the second centrifugation step is not recommended for the same reasons (Sletnes *et al*, 1992). Samples should not be repeatedly thawed and refrozen. Preliminary routine coagulation tests are helpful in eliminating undiagnosed coagulopathies and anticoagulant treatment.

Lupus anticoagulant testing

Classical findings for a LA are:
1 Prolongation of a phospholipid-dependent clotting test.
2 Demonstration of the presence of an inhibitor by mixing tests.
3 Demonstration of the phospholipid dependence of the inhibitor.

Two test systems of different principles should be employed to ensure that weak LA is detected and to improve specificity, though patients are regarded as having a LA if one test is positive. Clinical evidence based on associations with thrombosis suggests that the DRVVT has good utility and should be one of these tests. The other test will usually be an APTT using a reagent with proven LA sensitivity, a modified APTT, or a dilute prothrombin time. A mixing test may be used to detect an inhibitor and a confirmatory step (e.g. using a high phospholipid concentration, platelet neutralizing reagent or LA-insensitive reagent) is needed to demonstrate phospholipid dependence.

If the APTT is suggestive of LA but the DRVVT is negative, a confirmatory step in the APTT (or a further type of high specificity test employing screen and confirmatory assays) is needed to fulfil the criteria for LA.

Mixing tests are a criterion for LA and improve the specificity. However, they introduce a dilution factor and may make weak LA samples appear negative. In the absence of any other causes of prolonged clotting times, such samples should be considered LA positive if the screen and confirmatory tests on undiluted plasma give positive results (Clyne *et al*, 1993; Male *et al*, 2000; Thom *et al*, 2003; Moore & Savidge, 2006). Whenever possible, this should be confirmed by testing a fresh sample.

Cut-off values, calculations and quality control (QC) for LA tests. Given that there are differences in sensitivity and specificity between reagents (Denis-Magdelaine *et al*, 1995; Lawrie *et al*, 1999; Moore & Savidge, 2004) cut-off values for LA positivity should be specific for the given reagent and model of coagulometer (Lawrie *et al*, 1999; Gardiner *et al*, 2000). These values may be available from the manufacturer, but local validation is advised. Historically, laboratories have used the mean + 2·0 standard deviations (SD) (97·5th centile for normally distributed data) as a cut-off, but the recent International Society on Thrombosis and Haemostasis consensus document (Pengo *et al*, 2009) has recommended the 99th centile (mean + 2·3 SD for normally distributed data), which would improve specificity but reduce sensitivity. Most UK laboratories use the 97·5th centile. To estimate either with accuracy a large number of normal samples is needed and commercial frozen normal plasma sets, which must be sufficiently platelet-poor, may be useful in this respect. The inaccuracy of the reference interval estimation with small sample sizes is under-appreciated and sample sizes of 200 (Altman, 1991) and a minimum of 120 (Horowitz *et al*, 2008) have been recommended. If previously established cut-off values (manufacturer's value or different analyser) are available they may be validated in smaller numbers (20–60) of normal subjects (Horowitz *et al*, 2008).

A normal plasma pool (NPP) ($n \geq 6$) should be tested with each batch of samples and the patient screen and confirm results should be expressed as ratios against this. The method for calculating the degree of correction (in the confirm step) that has been recommended by the manufacturer should be used, provided that this takes into account the NPP clotting time. This should either employ the percentage correction of ratio = ((screen ratio − confirm ratio)/screen ratio) × 100 as previously described (Greaves *et al*, 2000), or a normalized test/confirm ratio = screen ratio/confirm ratio.

When reporting the results, the method, cut-off value, and an interpretation as LA-positive or LA-negative should be given.

Internal QC (IQC) must be performed with each batch of tests, using LA-negative and -positive plasmas. QC plasmas should be prepared in the same way as test samples. For the positive QC, plasma from a patient with well documented,

unequivocal APS and LA may be used. Commercial QC plasmas should be matched with the reagents and validated, as differences in buffering between plasmas and reagents can lead to erroneous results while platelet contamination of plasma pools will influence the sensitivity. Laboratories should also participate in an external quality assurance programme.

Recommendation

- **The DRVVT and one other test should be employed for LA detection (2C), and the patient regarded as having a LA if either test is positive.**
- **A confirmatory step (e.g. using a high phospholipid concentration, platelet neutralizing reagent or LA-insensitive reagent) is needed to demonstrate phospholipid dependence (1A).**

LA detection in patients receiving anticoagulants. LA testing is not recommended in patients receiving vitamin K antagonists (VKA) because exclusion of a LA is problematic whilst the international normalized ratio (INR) is in the therapeutic range. If it is thought to be helpful in determining the advisability of long-term anticoagulation, brief discontinuation of VKA therapy for diagnostic purposes is not a high risk strategy in most instances. When LA testing is required for patients receiving oral anticoagulants, the utility of the DRVVT is disputed (Jouhikainen, 1990; Olteanu *et al*, 2009) and tests performed on undiluted plasma may be misleading. Performing screening and confirmatory steps on equal volume mixtures of patient and normal plasma may be informative. If the screening step on the mixture is abnormal, this may be taken as grounds for considering that an inhibitor is present and the confirmatory step will demonstrate phospholipid dependence. Due to the dilution effect, negative testing in mixing studies does not exclude the presence of a LA. The taipan snake venom time is a useful secondary test to DRVVT in patients receiving oral anticoagulants, with high specificity for LA (Moore *et al*, 2003; Parmar *et al*, 2009), It can be used with a platelet neutralization procedure or ecarin time as confirmation.

LA tests should not be performed if the patient is receiving therapeutic doses of unfractionated heparin, because this may cause erroneous results (Schjetlein *et al*, 1993; Lawrie *et al*, 1999; Liestol *et al*, 2002). Low dose subcutaneous unfractionated heparin and low molecular weight heparin (LMWH) have less effect on the DRVVT and most commercial reagents contain a heparin neutralizang reagent sufficient to cover prophylactic doses. Platelet neutralization procedures should be avoided in samples containing heparin due to the potential for false positive LA results (Exner, 2000).

If positive results are obtained from aCL or anti-β_2GPI assays, these are sufficient for the diagnosis of APS.

Assessment of clotting factor levels in the presence of LA. Factor assays may yield misleading results, particularly those for intrinsic pathway factors based on 1-stage methods. Assays should be performed at several dilutions as poor parallelism indicates interference by the inhibitor and unreliable results. In this situation, using higher dilutions of the test sample can sometimes restore parallelism, but the standard curve must also be extended. Alternatively a LA-insensitive APTT reagent can be used for 1-stage assays. Another option is to use an assay system that is less dependent on phospholipid concentration, such as a 2-stage assay or certain chromogenic substrate assays. It should be recognized that some patients with factor inhibitors may also have a LA.

Solid phase aPL assays

Detailed guidance for the performance of aCL assays has recently been published (Pierangeli & Harris, 2008). Key features are the use of 10% adult bovine serum or fetal calf serum as a blocking agent and sample diluent and polyclonal (Pierangeli & Harris, 2008) or humanized monoclonal (Ichikawa *et al*, 1999) antibody calibrators with values in IgG or IgM antiphospholipid units (GPL units, MPL units).

Normal cut-off values should be established in healthy subjects using the 99th centile but it should be noted that the definition of APS used for research requires levels greater than the 99th centile or >40 GPL units.

Anti-β_2GPI assays have greater specificity than aCL, but are poorly standardized. The purity and oxidation status of the antigen and microtitre plate type are critical to ensure that the clinically relevant anti-β_2GPI epitopes are exposed; humanized monoclonal antibodies, such as HCAL and EY2C9, have been recommended as calibrants (Tincani *et al*, 2004; Reber *et al*, 2008) but are not commercially available. Assays have recently been developed that employ recombinant domain 1 of anti-β_2GPI, and may offer better sensitivity and specificity for clinical events, although more evidence is required.

A calibration curve and IQC should be employed in every assay run for both aCL and anti-β_2GPI assays. IQC may be performed using suitable normal or APS patient samples (local or commercial), or humanized monoclonal antibody preparations.

Which tests should be done?

LA is the most predictive test for thrombosis and the presence of IgG aCL or IgG anti-β_2GPI in those who are LA-positive increases the specificity. There is nothing to suggest that measuring IgM antibodies in patients with thrombosis adds useful information. Tests should be repeated after an interval of 12 weeks to demonstrate persistence.

Recommendation

- **When testing for aPL is indicated, testing for LA and for IgG antibodies to β_2GPI should be performed. The latter can be detected either by an IgG aCL ELISA or an IgG**

anti-β_2GPI ELISA (2C). An aCL ELISA may detect antibodies to other phosphoilipid binding proteins as well as anti-β_2GPI.
- **In patients with thrombosis, measuring IgM antibodies does not add useful information (2B).**
- **In patients with pregnancy morbidity, the role of IgM antibodies is unclear (2C).**
- **Testing for IgA antibodies is not recommended (1B).**
- **When assessing clinical significance account should be taken of whether the patient has LA, aCL/anti-β_2GPI, or both and of the isotype and titre in the solid phase tests (1B).**

Who should be tested for aPL and how should this affect management of patients

Incidental finding of aPL

Incidental detection of aPL is common, e.g. in the Leiden thrombophilia study, a population-based case control study of VTE, LA was present in 0·9% of unaffected controls (and 3·1% of cases) and anti-β_2GPI in 3·4% of controls (and 7·5% of patients) (de Groot et al, 2005). Even when persistent, incidental antibodies have been thought to be associated with a low rate of thrombosis, e.g. 36 (6·5%) of 552 normal blood donors were found to have IgG aCL (eight remained positive for 9 months) but none had thrombosis during the 12 month follow-up (0% 95% CI 0–9·7%) (Vila et al, 1994). In a larger study, 178 asymptomatic carriers of aPL were followed up for 36 months and no episode of thrombosis was detected (0% 95% CI 0–2·0%) (Giron-Gonzalez et al, 2004). However, a recent publication identified 104 subjects that were triple positive for LA, aCL and anti-β_2GPI, and follow-up for a mean of 4·5 years identified 25 first thromboembolic events (5·3% per year)(Pengo et al, 2011). Aspirin did not significantly affect the incidence of thromboembolism, consistent with a randomized trial in which thromboprophylaxis with aspirin was ineffective: in 98 individuals with aPL but no clinical manifestations randomized to receive aspirin ($n = 48$) or placebo ($n = 50$) the acute thrombosis incidence rates were 2·75 per 100 patient-years for aspirin-treated subjects and 0 per 100 patient-years for the placebo-treated subjects ($P = 0.83$) (Erkan et al, 2007).

Recommendation

- **We recommend that primary thromboprophylaxis should not be used in those incidentally found to have aPL (2B).**

Which patients with venous thrombosis should be tested for aPL and how should the result affect management?

Warfarin therapy carries a substantial risk of bleeding. Although the risk is greatest in the first weeks, it persists for the duration of exposure. Initial treatment is for at least 3 months, thereafter decisions regarding the continuation of treatment long-term after an episode of VTE should be based on an individual assessment of the risk-benefit ratio. The risk of recurrence is significantly higher after an unprovoked event (Iorio et al, 2010). Retrospective studies have shown a high incidence of thrombosis recurrence in patients with aPL (Rosove & Brewer, 1992; Khamashta et al, 1995; Krnic-Barrie et al, 1997). In these studies, 80/147 (Khamashta et al, 1995), 39/70 (Rosove & Brewer, 1992) and 23/61 (Krnic-Barrie et al, 1997) had venous thrombosis. In the prospective Duration of Anticoagulation (DURAC) study a single aCL positive test doubled the risk of a recurrence (Schulman et al, 1998).

In patients with venous thrombosis, a finite duration of treatment is recommended for patients with a transient risk factor but long-term anticoagulation is considered in those with an unprovoked event (Kearon et al, 2008). We do not recommend testing for aPL in patients with venous thrombosis due to a transient risk factor as we do not think there is sufficient evidence to recommend long-term anticoagulation even if the patient has aPL. If it is decided to stop anticoagulation after unprovoked proximal DVT or PE, testing for aPL is indicated as their presence will increase the risk of recurrence favouring long-term anticoagulation.

Recommendation

- **We recommend testing for aPL in patients with unprovoked proximal DVT or PE after stopping anticoagulation (for at least 7 d) as the presence of aPL will influence the balance of risks and benefits and support long-term anticoagulant therapy (2B).**

Which patients with ischaemic stroke should be tested for aPL and how should the result affect management?

As a result of retrospective and observational studies it was thought that stroke associated with aPL carried a high risk of recurrence (with the likelihood of consequent permanent disability or death) and should be treated with long-term warfarin (Rosove & Brewer, 1992; Khamashta et al, 1995; Krnic-Barrie et al, 1997). The Antiphospholipid Antibodies and Stroke Study (APASS) (Levine et al, 2004) was a prospective cohort study within the Warfarin versus Aspirin Recurrent Stroke Study (WARSS), a randomized double-blind trial comparing warfarin (INR 1·4–2·8) with aspirin. 720 out of 1770 stroke patients (41%) were aPL positive (13% LA, 20% aCL, 7% both) and aPL did not predict recurrence: OR 0·99 (0·75–1·31) and 0·94 (0·70–1·28) for the patients on warfarin and aspirin, respectively. It should be noted that tests for aPL were only performed on a single occasion and that IgG aCL > 21 GPL units was regarded as positive. For patients with a single positive aPL test result and prior stroke, aspirin and moderate-intensity warfarin

appear equally effective for preventing recurrent stroke. We have no high quality evidence for young patients with stroke who have APS according to the Miyakis et al (2006) criteria (Table I). The cohort studies previously referred to suggest that young patients (<50 years) with ischaemic stroke and APS may be at high risk of recurrence (patients who are triple positive for LA, aCL and anti-β_2GPI have the highest risk), and that anticoagulation with warfarin should be considered, but there is no strong evidence that it is more effective than aspirin. A small retrospective study followed eight patients with APS treated with aspirin for a median of 9 years after an ischaemic stroke: there were two recurrences in a total of 58 patient years on aspirin to give a recurrence rate of 3·5% per year, similar to the general stroke population (Derksen et al, 2003). In a further small study, 20 ischaemic stroke patients with aPL were randomized to either aspirin alone (n = 11) or aspirin plus warfarin (target INR 2–3) (Okuma et al, 2010). The cumulative incidence of stroke in patients with antiplatelet treatment only was statistically significantly higher than that in patients receiving the combination of antiplatelet and anticoagulation therapy. The authors suggested a larger study with more patients would be warranted. In the general stroke population, aspirin plus dipyridamole, or clopidogrel alone, are superior to aspirin alone.

Recommendations

- **Routine screening for aPL in patients with ischaemic stroke is not warranted (1B).**
- **Young adults (<50 years) with ischaemic stroke should be screened for aPL (2C).**
- **For unselected stroke patients with a single positive aPL test result, antiplatelet therapy and warfarin are equally effective for preventing recurrent stroke (1B) and antiplatelet therapy is preferred on grounds of convenience.**
- **Young adults (<50 years) with ischaemic stroke and APS may be at high risk of recurrence and cohort studies suggest that anticoagulation with warfarin should be considered, but there is no strong evidence that it is better than antiplatelet therapy (2C).**

Catastrophic APS

CAPS is an acute onset, life-threatening cause of multi-organ failure (Cervera et al, 2009). It is a rare condition that may complicate established APS or present de novo. There are no data from randomized trials to inform treatment, which is based upon the thrombotic features and autoimmune background. Combinations of treatments are typically used including anticoagulation with heparin/warfarin. Immunomodulatory therapies including plasmaphaeresis, intravenous human IgG, corticosteroids and rituximab have been employed.

Anticoagulation in APS

As in all subjects with thrombosis, attention should be paid to modifiable risk factors such as smoking, obesity and exogenous female hormone use. Although there is developing interest in, and some rationale for, use of alternatives to anticoagulant drugs to reduce thrombosis risk in APS, specifically statins (Ferrara et al, 2003, 2004) and hydroxychloroquine (Edwards et al, 1997; Espinola et al, 2002; Rand et al, 2008), their use remains experimental at present.

Intensity of anticoagulation in APS. A retrospective study of 147 patients (54% with venous thrombosis) suggested that a target INR of 3·5 was preferable to a target INR of 2·5 (Khamashta et al, 1995). Two subsequent prospective randomized trials have challenged this. Crowther et al (2003) randomized 114 patients with aPL and thrombosis (76% venous, 24% arterial) to a target INR of 2·5 or 3·5 and followed them for a mean of 2·7 years. Recurrences were 2/58 (3·4%) in the low intensity group and 6/56 (10·7%) in the high intensity group. For venous thrombosis the rates were 1/45 (2·2%) and 3/42 (7·1%), respectively. Finazzi et al (2005) randomized 109 patients with aPL and thrombosis (60% venous only, 31% arterial only, 9% both) to a target INR of 2–3 or 3–4·5 and followed them for a median of 3·6 years. Recurrences were 3/52 (5·8%) in the low intensity group and 6/54 (11·1%) in the high intensity group.

Recommendation

- **The target INR for VKA therapy in APS should normally be 2·5 (target range 2·0–3·0) (1A).**

Monitoring oral anticoagulants in patients with a lupus anticoagulant

The majority of patients (>95%) with APS have a normal prothrombin time (PT) in the absence of other coagulopathies or anticoagulant use. When the PT is prolonged, it is sometimes due to hypoprothrombinaemia, but it has been suggested that the PT/INR may be falsely increased by interference of LA with the phospholipid component of the PT reagent, particularly where recombinant tissue factor is employed and purified phospholipids are used for relipidation. Certain reagents, such as Innovin and Thromborel R (Tripodi et al, 2001) appear to be more sensitive to LA. Where the baseline PT is elevated, alternative, LA-insensitive PT reagents should be employed. Point-of-care devices should be used with caution for INR determination in APS (Briggs et al, 2008; Perry et al, 2010). Most manufacturers list APS as a specific exclusion to their use. In rare patients with prolongation of the baseline PT (one study (Moore et al, 2005) found this in 4·3% of cases using Innovin, n = 400), which causes difficulty in establishing the true degree of anticoagula-

tion; amidolytic factor X (FX) assays may be helpful (Tripodi *et al*, 2001; Moore *et al*, 2003). A therapeutic range of approximately 20–40% FX corresponds to a therapeutic INR in LA-negative patients (Rosborough *et al*, 2010).

Recommendations

- **A baseline PT should be performed; if this is prolonged, an alternative PT reagent for which the baseline is normal should be used (1C).**
- **If there are problems identifying a suitable PT system for VKA control, the use of an amidolytic FX assay could be considered (2C).**

Which patients with obstetric complications should be tested for aPL and how should the result affect management?

The investigation and treatment of women with recurrent pregnancy loss is covered in an RCOG guideline (http://www.rcog.org.uk/files/rcog-corp/GTG17recurrentmiscarriage.pdf). The pregnant state may have some effect on tests for aPL, suggesting that investigation should be pursued between pregnancies where possible (Topping *et al*, 1999).

Antithrombotic interventions are used to reduce the incidence of pregnancy complications. In APS this management is supported by clinical trials (Kutteh & Ermel, 1996; Rai *et al*, 1997) and systematic review (Empson *et al*, 2005), which reported that unfractionated heparin (UFH) in combination with low dose aspirin reduces the incidence of pregnancy loss in women with a history of recurrent loss. Although data are limited, increasing the dose of UFH (combined with low dose aspirin) does not appear to decrease the risk of pregnancy loss further (Kutteh & Ermel, 1996; Empson *et al*, 2005). Low dose aspirin therapy alone has not been shown to reduce pregnancy loss compared with routine care or placebo (Cowchock & Reece, 1997; Tulppala *et al*, 1997; Pattison *et al*, 2000; Empson *et al*, 2005). In contrast to UFH, the combination of LMWH and low dose aspirin did not result in a reduced rate of pregnancy loss compared with aspirin alone (Farquharson *et al*, 2002; Empson *et al*, 2005; Laskin *et al*, 2009). Although LMWH has replaced UFH in pregnancy because of a more favourable safety profile and once daily dosing (Greer & Nelson-Piercy, 2005) there are few data comparing LMWH and UFH. However, in two small pilot studies the combination of LMWH and low dose aspirin appeared equivalent to UFH and low dose aspirin in preventing recurrent pregnancy loss (Stephenson *et al*, 2004; Noble *et al*, 2005).

Although there is limited evidence of efficacy, LMWH has largely replaced UFH in obstetric practice for treatment of recurrent miscarriage in APS because of safety and ease of use. Despite inclusion of fetal death placental insufficiency and severe early pre-eclampsia in the consensus criteria for diagnosis of APS, the data supporting the associations have been conflicting to date and there is a lack of robust evidence to guide treatment (Branch, 2011).

Low dose aspirin is established for prevention of FGR and pre-eclampsia and is appropriate to use in women with APS and a history of these complications. However there is a lack of evidence to demonstrate that adding UFH or LMWH carries additional benefit for secondary prevention of these late pregnancy complications in women with APS. Thus, while such therapy may be considered, based on an extrapolation from recurrent pregnancy loss evidence, at present this practice is not supported by the limited evidence available.

An RCOG guideline recommends that women with previous thrombosis and APS should be offered both antenatal and 6 weeks of post-partum thromboprophylaxis and that women with persistent aPL with no previous VTE and no other risk factors or fetal indications for LMWH may be managed with close surveillance antenatally but should be considered for LMWH for 7 d postpartum (http://www.rcog.org.uk/files/rcog-corp/GTG37aReducingRiskThrombosis.pdf).

Recommendations

- **Women with recurrent pregnancy loss (≥ 3 pregnancy losses) before 10 weeks gestation should be screened for aPL (1B).**
- **For women with APS with recurrent (≥ 3) pregnancy loss, antenatal administration of heparin combined with low dose aspirin is recommended throughout pregnancy (1B). In general, treatment should begin as soon as pregnancy is confirmed.**
- **For women with APS and a history of pre-eclampsia or FGR, low dose aspirin is recommended.**
- **Women with aPL should be considered for post-partum thromboprophylaxis (1B).**

Disclaimer

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References

Agar, C., van Os, G.M., Morgelin, M., Sprenger, R. R., Marquart, J.A., Urbanus, R.T., Derksen, R. H., Meijers, J.C. & de Groot, P.G. (2010) β_2-Glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. *Blood*, **116**, 1336–1343.

Altman, D.G. (1991) Practical Statistics for Medical Research. Chapman & Hall, London.

Amengual, O., Atsumi, T., Khamashta, M.A. & Hughes, G.R. (1998) The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thrombosis and Haemostasis*, **79**, 276–281.

Atsumi, T., Khamashta, M.A., Amengual, O., Donohoe, S., Mackie, I., Ichikawa, K., Koike, T. & Hughes, G.R. (1998a) Binding of anticardiolipin antibodies to protein C via beta2-glycoprotein I (beta2-GPI): a possible mechanism in the inhibitory effect of antiphospholipid antibodies on the protein C system. *Clinical and Experimental Immunology*, **112**, 325–333.

Atsumi, T., Khamashta, M.A., Andujar, C., Leandro, M.J., Amengual, O., Ames, P.R. & Hughes, G.R. (1998b) Elevated plasma lipoprotein(a) level and its association with impaired fibrinolysis in patients with antiphospholipid syndrome. *Journal of Rheumatology*, **25**, 69–73.

Bertolaccini, M.L., Atsumi, T., Escudero Contreras, A., Khamashta, M.A. & Hughes, G.R. (2001) The value of IgA antiphospholipid testing for diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosus. *Journal of Rheumatology*, **28**, 2637–2643.

Bevers, E.M., Galli, M., Barbui, T., Comfurius, P. & Zwaal, R.F. (1991) Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thrombosis and Haemostasis*, **66**, 629–632.

Blank, M., Cohen, J., Toder, V. & Shoenfeld, Y. (1991) Induction of anti-phospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 3069–3073.

Bongard, O., Reber, G., Bounameaux, H. & de Moerloose, P. (1992) Anticardiolipin antibodies in acute venous thromboembolism [letter]. *Thrombosis and Haemostasis*, **67**, 724.

Branch, W. (2011) Report of the Obstetric APS Task Force: 13th International Congress on Antiphospholipid Antibodies, 13th April 2010. *Lupus*, **20**, 158–164.

Branch, D.W. & Rodgers, G.M. (1993) Induction of endothelial cell tissue factor activity by sera from patients with antiphospholipid syndrome: a possible mechanism of thrombosis. *American Journal of Obstetrics and Gynecology*, **168**, 206–210.

Branch, D.W., Porter, T.F., Rittenhouse, L., Caritis, S., Sibai, B., Hogg, B., Lindheimer, M.D., Klebanoff, M., MacPherson, C., VanDorsten, J.P., Landon, M., Paul, R., Miodovnik, M., Meis, P. & Thurnau, G. (2001) Antiphospholipid antibodies in women at risk for preeclampsia. *American Journal of Obstetrics and Gynecology*, **184**, 825–832; discussion 832–824.

Briggs, C., Guthrie, D., Hyde, K., Mackie, I., Parker, N., Popek, M., Porter, N. & Stephens, C. (2008) Guidelines for point-of-care testing: haematology. *British Journal of Haematology*, **142**, 904–915.

Buckingham, K.L. & Chamley, L.W. (2009) A critical assessment of the role of antiphospholipid antibodies in infertility. *Journal of Reproductive Immunology*, **80**, 132–145.

Cavestro, C., Micca, G., Molinari, F., Bazzan, M., C, D.I.P., Aloi, R., Pedemonte, E., Iannini, R., Frigeri, M.C. & Roccatello, D. (2011) Migraineurs show a high prevalence of antiphospholipid antibodies. *Journal of Thrombosis and Haemostasis*, **9**, 1350–1354.

Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., Shoenfeld, Y., Camps, M.T., Jacobsen, S., Lakos, G., Tincani, A., Kontopoulou-Griva, I., Galeazzi, M., Meroni, P.L., Derksen, R.H., de Groot, P.G., Gromnica-Ihle, E., Baleva, M., Mosca, M., Bombardieri, S., Houssiau, F., Gris, J.C., Quere, I., Hachulla, E., Vasconcelos, C., Roch, B., Fernandez-Nebro, A., Boffa, M.C., Hughes, G.R. & Ingelmo, M. (2002) Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis and Rheumatism*, **46**, 1019–1027.

Cervera, R., Bucciarelli, S., Plasin, M.A., Gomez-Puerta, J.A., Plaza, J., Pons-Estel, G., Shoenfeld, Y., Ingelmo, M. & Espinos, G. (2009) Catastrophic antiphospholipid syndrome (CAPS): descriptive analysis of a series of 280 patients from the "CAPS Registry". *Journal of Autoimmunity*, **32**, 240–245.

Chamley, L.W., Duncalf, A.M., Mitchell, M.D. & Johnson, P.M. (1998) Action of anticardiolipin and antibodies to beta2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. *Lancet*, **352**, 1037–1038.

Clyne, L.P., Yen, Y., Kriz, N.S. & Breitenstein, M. G. (1993) The lupus anticoagulant. High incidence of 'negative' mixing studies in a human immunodeficiency virus-positive population. *Archives of Pathology and Laboratory Medicine*, **117**, 595–601.

Cowchock, S. & Reece, E.A. (1997) Do low-risk pregnant women with antiphospholipid antibodies need to be treated? Organizang Group of the Antiphospholipid Antibody Treatment Trial. *American Journal of Obstetrics and Gynecology*, **176**, 1099–1100.

Crowther, M.A., Ginsberg, J.S., Julian, J., Denburg, J., Hirsh, J., Douketis, J., Laskin, C., Fortin, P., Anderson, D., Kearon, C., Clarke, A., Geerts, W., Forgie, M., Green, D., Costantini, L., Yacura, W., Wilson, S., Gent, M. & Kovacs, M.J. (2003) A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. *New England Journal of Medicine*, **349**, 1133–1138.

de Groot, P.G. & Meijers, J.C. (2011) β_2-Glycoprotein I: evolution, structure and function. *Journal of Thrombosis and Haemostasis*, **9**, 1275–1284.

de Groot, P.G., Lutters, B., Derksen, R.H., Lisman, T., Meijers, J.C. & Rosendaal, F.R. (2005) Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. *Journal of Thrombosis and Haemostasis*, **3**, 1993–1997.

de Laat, H.B., Derksen, R.H., Urbanus, R.T., Roest, M. & de Groot, P.G. (2004) β_2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. *Blood*, **104**, 3598–3602.

de Laat, B., Derksen, R.H., Urbanus, R.T. & de Groot, P.G. (2005) IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. *Blood*, **105**, 1540–1545.

Denis-Magdelaine, A., Flahault, A. & Verdy, E. (1995) Sensitivity of sixteen APTT reagents for the presence of lupus anticoagulants. *Haemostasis*, **25**, 98–105.

Derksen, R.H., de Groot, P.G. & Kappelle, L.J. (2003) Low dose aspirin after ischemic stroke associated with antiphospholipid syndrome. *Neurology*, **61**, 111–114.

Edwards, M.H., Pierangeli, S., Liu, X., Barker, J.H., Anderson, G. & Harris, E.N. (1997) Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice. *Circulation*, **96**, 4380–4384.

Empson, M.B., Lassere, M., Craig, J.C. & Scott, J. R. (2005) Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database of Systematic Reviews*, Issue 2. Art. No.: CD002859. DOI: 10.1002/14651858.CD002859.pub2.

Erkan, D., Harrison, M.J., Levy, R., Peterson, M., Petri, M., Sammaritano, L., Unalp-Arida, A., Vilela, V., Yazici, Y. & Lockshin, M.D. (2007) Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. *Arthritis and Rheumatism*, **56**, 2382–2391.

Espinola, R.G., Pierangeli, S.S., Gharavi, A.E. & Harris, E.N. (2002) Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies. *Thrombosis and Haemostasis*, **87**, 518–522.

Exner, T. (2000) Conceptions and misconceptions in testing for lupus anticoagulants. *Journal of Autoimmunity*, **15**, 179–183.

Farquharson, R.G., Quenby, S. & Greaves, M. (2002) Antiphospholipid syndrome in

pregnancy: a randomized, controlled trial of treatment. *Obstetrics and Gynecology*, **100**, 408–413.

Favaloro, E.J. (2007) Preanalytical variables in coagulation testing. *Blood Coagulation and Fibrinolysis*, **18**, 86–89.

Ferrara, D.E., Liu, X., Espinola, R.G., Meroni, P.L., Abukhalaf, I., Harris, E.N. & Pierangeli, S.S. (2003) Inhibition of the thrombogenic and inflammatory properties of antiphospholipid antibodies by fluvastatin in an in vivo animal model. *Arthritis and Rheumatism*, **48**, 3272–3279.

Ferrara, D.E., Swerlick, R., Casper, K., Meroni, P. L., Vega-Ostertag, M.E., Harris, E.N. & Pierangeli, S.S. (2004) Fluvastatin inhibits up-regulation of tissue factor expression by antiphospholipid antibodies on endothelial cells. *Journal of Thrombosis and Haemostasis*, **2**, 1558–1563.

Finazzi, G., Marchioli, R., Brancaccio, V., Schinco, P., Wisloff, F., Musial, J., Baudo, F., Berrettini, M., Testa, S., D'Angelo, A., Tognoni, G. & Barbui, T. (2005) A randomized clinical trial of high-intensity warfarin vs. conventional antithrombotic therapy for the prevention of recurrent thrombosis in patients with the antiphospholipid syndrome (WAPS). *Journal of Thrombosis and Haemostasis*, **3**, 848–853.

Galli, M., Comfurius, P., Maassen, C., Hemker, H. C., de Baets, M.H., van Breda Vriesman, P.J., Barbui, T., Zwaal, R.F. & Bevers, E.M. (1990) Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor [see comments]. *Lancet*, **335**, 1544–1547.

Galli, M., Luciani, D., Bertolini, G. & Barbui, T. (2003a) Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. *Blood*, **102**, 2717–2723.

Galli, M., Luciani, D., Bertolini, G. & Barbui, T. (2003b) Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. *Blood*, **101**, 1827–1832.

Galli, M., Borrelli, G., Jacobsen, E.M., Marfisi, R. M., Finazzi, G., Marchioli, R., Wisloff, F., Marziali, S., Morboeuf, O. & Barbui, T. (2007) Clinical significance of different antiphospholipid antibodies in the WAPS (warfarin in the antiphospholipid syndrome) study. *Blood*, **110**, 1178–1183.

Gardiner, C., Mackie, I.J., Malia, R.G., Jones, D. W., Winter, M., Leeming, D., Taberner, D.A., Machin, S.J. & Greaves, M. (2000) The importance of locally derived reference ranges and standardized calculation of dilute Russell's viper venom time results in screening for lupus anticoagulant. *British Journal of Haematology*, **111**, 1230–1235.

Giannakopoulos, B. & Krilis, S.A. (2009) How I treat the antiphospholipid syndrome. *Blood*, **114**, 2020–2030.

Giannakopoulos, B., Passam, F., Rahgozar, S. & Krilis, S.A. (2007) Current concepts on the

pathogenesis of the antiphospholipid syndrome. *Blood*, **109**, 422–430.

Giannakopoulos, B., Passam, F., Ioannou, Y. & Krilis, S.A. (2009) How we diagnose the antiphospholipid syndrome. *Blood*, **113**, 985–994.

Ginsberg, J.S., Brill-Edwards, P., Johnston, M., Denburg, J.A., Andrew, M., Burrows, R.F., Bensen, W., Cividino, A. & Long, A.A. (1992) Relationship of antiphospholipid antibodies to pregnancy loss in patients with systemic lupus erythematosus: a cross-sectional study. *Blood*, **80**, 975–980.

Ginsburg, K.S., Liang, M.H., Newcomer, L., Goldhaber, S.Z., Schur, P.H., Hennekens, C.H. & Stampfer, M.J. (1992) Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis [see comments]. *Annals of Internal Medicine*, **117**, 997–1002.

Girardi, G., Redecha, P. & Salmon, J.E. (2004) Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nature Medicine*, **10**, 1222–1226.

Giron-Gonzalez, J.A., Garcia del Rio, E., Rodriguez, C., Rodriguez-Martorell, J. & Serrano, A. (2004) Antiphospholipid syndrome and asymptomatic carriers of antiphospholipid antibody: prospective analysis of 404 individuals. *Journal of Rheumatology*, **31**, 1560–1567.

Greaves, M., Cohen, H., MacHin, S. & Mackie, I. (2000) Guidelines on the investigation and management of the antiphospholipid syndrome. *British Journal Haematology*, **109**, 704–715.

Greer, I.A. & Nelson-Piercy, C. (2005) Low-molecular-weight heparins for thromboprophylaxis and treatment of venous thromboembolism in pregnancy: a systematic review of safety and efficacy. *Blood*, **106**, 401–407.

Heinzlef, O., Weill, B., Johanet, C., Sazdovitch, V., Caillat-Zucman, S., Tournier-Lasserve, E. & Roullet, E. (2002) Anticardiolipin antibodies in patients with multiple sclerosis do not represent a subgroup of patients according to clinical, familial, and biological characteristics. *Journal of Neurology, Neurosurgery and Psychiatry*, **72**, 647–649.

Horowitz, G.L., Altaie, S., Boyd, J.C., Ceriotti, F., Garg, U., Horn, P., Pesce, A., Sine, H.E. & Zakowski, J. (2008) Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline – Third Edition. CLSI document C28-A3c. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Hughes, G.R. (2003) Migraine, memory loss, and "multiple sclerosis". Neurological features of the antiphospholipid (Hughes') syndrome. *Postgraduate Medical Journal*, **79**, 81–83.

Hughes, G.R. & Khamashta, M.A. (2003) Seronegative antiphospholipid syndrome. *Annals of the Rheumatic Diseases*, **62**, 1127.

Ichikawa, K., Tsutsumi, A., Atsumi, T., Matsuura, E., Kobayashi, S., Hughes, G.R., Khamashta, M. A. & Koike, T. (1999) A chimeric antibody with the human gamma1 constant region as a putative standard for assays to detect IgG beta2-glycoprotein I-dependent anticardiolipin and anti-

beta2-glycoprotein I antibodies. *Arthritis and Rheumatism*, **42**, 2461–2470.

Iorio, A., Kearon, C., Filippucci, E., Marcucci, M., Macura, A., Pengo, V., Siragusa, S. & Palareti, G. (2010) Risk of recurrence after a first episode of symptomatic venous thromboembolism provoked by a transient risk factor: a systematic review. *Archives of Internal Medicine*, **170**, 1710–1716.

Jouhikainen, T. (1990) Detection of lupus anticoagulant by means of dilute Russell's viper venom time is affected by oral anticoagulant therapy. *Blood Coagulation and Fibrinolysis*, **1**, 627–632.

Kearon, C., Kahn, S.R., Agnelli, G., Goldhaber, S., Raskob, G.E. & Comerota, A.J. (2008) Antithrombotic therapy for venous thromboembolic disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*, **133**, 454S–545S.

Khamashta, M.A., Cuadrado, M.J., Mujic, F., Taub, N.A., Hunt, B.J. & Hughes, G.R. (1995) The management of thrombosis in the antiphospholipid-antibody syndrome [see comments]. *New England Journal of Medicine*, **332**, 993–997.

Krnic-Barrie, S., O'Connor, C.R., Looney, S.W., Pierangeli, S.S. & Harris, E.N. (1997) A retrospective review of 61 patients with antiphospholipid syndrome. Analysis of factors influencing recurrent thrombosis. *Archives of Internal Medicine*, **157**, 2101–2108.

Kutteh, W.H. & Ermel, L.D. (1996) A clinical trial for the treatment of antiphospholipid antibody-associated recurrent pregnancy loss with lower dose heparin and aspirin. *American Journal of Reproductive Immunology*, **35**, 402–407.

Laskin, C.A., Bombardier, C., Hannah, M.E., Mandel, F.P., Ritchie, J.W., Farewell, V., Farine, D., Spitzer, K., Fielding, L., Soloninka, C.A. & Yeung, M. (1997) Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *New England Journal of Medicine*, **337**, 148–153.

Laskin, C.A., Spitzer, K.A., Clark, C.A., Crowther, M.R., Ginsberg, J.S., Hawker, G.A., Kingdom, J. C., Barrett, J. & Gent, M. (2009) Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA Trial. *Journal of Rheumatology*, **36**, 279–287.

Lawrie, A.S., Mackie, I.J., Purdy, G. & Machin, S.J. (1999) The sensitivity and specificity of commercial reagents for the detection of lupus anticoagulant show marked differences in performance between photo-optical and mechanical coagulometers. *Thrombosis and Haemostasis*, **81**, 758–762.

Levine, S.R., Brey, R.L., Tilley, B.C., Thompson, J. L., Sacco, R.L., Sciacca, R.R., Murphy, A., Lu, Y., Costigan, T.M., Rhine, C., Levin, B., Triplett, D.A. & Mohr, J.P. (2004) Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. *JAMA*, **291**, 576–584.

Liestol, S., Jacobsen, E.M. & Wisloff, F. (2002) Dilute prothrombin time-based lupus ratio test.

Integrated LA testing with recombinant tissue thromboplastin. *Thrombosis Research*, **105**, 177–182.

Lim, W., Crowther, M.A. & Eikelboom, J.W. (2006) Management of antiphospholipid antibody syndrome: a systematic review. *JAMA*, **295**, 1050–1057.

Male, C., Lechner, K., Speiser, W. & Pabinger, I. (2000) Transient lupus anticoagulants in children: stepwise disappearance of diagnostic features. *Thrombosis and Haemostasis*, **83**, 174–175.

Malia, R.G., Kitchen, S., Greaves, M. & Preston, F. E. (1990) Inhibition of activated protein C and its cofactor protein S by antiphospholipid antibodies [see comments]. *British Journal Haematology*, **76**, 101–107.

McNeil, H.P., Simpson, R.J., Chesterman, C.N. & Krilis, S.A. (1990) Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4120–4124.

Miyakis, S., Lockshin, M.D., Atsumi, T., Branch, D.W., Brey, R.L., Cervera, R., Derksen, R.H., de Groot, P.G., Koike, T., Meroni, P.L., Reber, G., Shoenfeld, Y., Tincani, A., Vlachoyiannopoulos, P.G. & Krilis, S.A. (2006) International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *Journal of Thrombosis and Haemostasis*, **4**, 295–306.

Montalban, J., Cervera, R., Font, J., Ordi, J., Vianna, J., Haga, H.J., Tintore, M., Khamashta, M.A. & Hughes, G.R. (1992) Lack of association between anticardiolipin antibodies and migraine in systemic lupus erythematosus. *Neurology*, **42**, 681–682.

Moore, G.W. & Savidge, G.F. (2004) Heterogeneity of Russell's viper venom affects the sensitivity of the dilute Russell's viper venom time to lupus anticoagulants. *Blood Coagulation and Fibrinolysis*, **15**, 279–282.

Moore, G.W. & Savidge, G.F. (2006) The dilution effect of equal volume mixing studies compromises confirmation of inhibition by lupus anticoagulants even when mixture specific reference ranges are applied. *Thrombosis Research*, **118**, 523–528.

Moore, G.W., Smith, M.P. & Savidge, G.F. (2003) The Ecarin time is an improved confirmatory test for the Taipan snake venom time in warfarinized patients with lupus anticoagulants. *Blood Coagulation and Fibrinolysis*, **14**, 307–312.

Moore, G.W., Rangarajan, S., Holland, L.J., Henley, A. & Savidge, G.F. (2005) Low frequency of elevated prothrombin times in patients with lupus anticoagulants when using a recombinant thromboplastin reagent: implications for dosing and monitoring of oral anticoagulant therapy. *British Journal of Biomedical Science*, **62**, 15–18; quiz 47.

Naess, I.A., Christiansen, S.C., Cannegieter, S.C., Rosendaal, F.R. & Hammerstroem, J. (2006) A prospective study of anticardiolipin antibodies as a risk factor for venous thrombosis in a general population (the HUNT study). *Journal of Thrombosis and Haemostasis*, **4**, 44–49.

Nelson, S.M. & Greer, I.A. (2008) The potential role of heparin in assisted conception. *Human Reproduction Update*, **14**, 623–645.

Noble, L.S., Kutteh, W.H., Lashey, N., Franklin, R. D. & Herrada, J. (2005) Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low-molecular-weight heparin versus unfractionated heparin. *Fertility and Sterility*, **83**, 684–690.

Oger, E., Lernyer, C., Dueymes, M., Le Moigne, E., Bressolette, L., Escoffre, M., Youinou, P. & Mottier, D. (1997) Association between IgM anticardiolipin antibodies and deep venous thrombosis in patients without systemic lupus erythematosus. *Lupus*, **6**, 455–461.

Oku, K., Atsumi, T., Bohgaki, M., Amengual, O., Kataoka, H., Horita, T., Yasuda, S. & Koike, T. (2009) Complement activation in patients with primary antiphospholipid syndrome. *Annals of the Rheumatic Diseases*, **68**, 1030–1035.

Okuma, H., Kitagawa, Y., Yasuda, T., Tokuoka, K. & Takagi, S. (2010) Comparison between single antiplatelet therapy and combination of antiplatelet and anticoagulation therapy for secondary prevention in ischemic stroke patients with antiphospholipid syndrome. *International Journal of Medical Sciences*, **7**, 15–18.

Olteanu, H., Downes, K.A., Patel, J., Praprotnik, D. & Sarode, R. (2009) Warfarin does not interfere with lupus anticoagulant detection by dilute Russell's viper venom time. *Clinical laboratory*, **55**, 138–142.

Opatrny, L., David, M., Kahn, S.R., Shrier, I. & Rey, E. (2006) Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a meta-analysis. *Journal of Rheumatology*, **33**, 2214–2221.

Parmar, K., Lefkou, E., Doughty, H., Connor, P. & Hunt, B.J. (2009) The utility of the Taipan snake venom assay in assessing lupus anticoagulant status in individuals receiving or not receiving an oral vitamin K antagonist. *Blood Coagulation and Fibrinolysis*, **20**, 271–275.

Pattison, N.S., Chamley, L.W., Birdsall, M., Zanderigo, A.M., Liddell, H.S. & McDougall, J. (2000) Does aspirin have a role in improving pregnancy outcome for women with the antiphospholipid syndrome? A randomized controlled trial. *American Journal of Obstetrics and Gynecology*, **183**, 1008–1012.

Pengo, V., Tripodi, A., Reber, G., Rand, J.H., Ortel, T.L., Galli, M. & De Groot, P.G. (2009) Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *Journal of Thrombosis and Haemostasis*, **7**, 1737–1740.

Pengo, V., Ruffatti, A., Legnani, C., Gresele, P., Barcellona, D., Erba, N., Testa, S., Marongiu, F., Bison, E., Denas, G., Banzato, A., Padayattil Jose, S. & Iliceto, S. (2010) Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. *Journal of Thrombosis and Haemostasis*, **8**, 237–242.

Pengo, V., Ruffatti, A., Legnani, C., Testa, S., Fierro, T., Marongiu, F., De Micheli, V., Gresele, P., Tonello, M., Ghirarduzzi, A., Bison, E., Denas, G., Banzato, A., Padayattil Jose, S. & Iliceto, S. (2011) Incidence of a first thromboembolic event in asymptomatic carriers of high risk antiphospholipid antibody profile: a multicenter prospective study. *Blood*, **118**, 4714–4718.

Perry, D.J., Fitzmaurice, D.A., Kitchen, S., Mackie, I.J. & Mallett, S. (2010) Point-of-care testing in haemostasis. *British Journal of Haematology*, **150**, 501–514.

Pierangeli, S.S. & Harris, E.N. (2008) A protocol for determination of anticardiolipin antibodies by ELISA. *Nature Protocols*, **3**, 840–848.

Rai, R.S., Clifford, K., Cohen, H. & Regan, L. (1995) High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Human Reproduction*, **10**, 3301–3304.

Rai, R., Cohen, H., Dave, M. & Regan, L. (1997) Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ*, **314**, 253–257.

Rand, J.H., Wu, X.X., Andree, H.A., Ross, J.B., Rusinova, E., Gascon-Lema, M.G., Calandri, C. & Harpel, P.C. (1998) Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin-V binding to phospholipids: a "lupus procoagulant" phenomenon. *Blood*, **92**, 1652–1660.

Rand, J.H., Wu, X.X., Quinn, A.S., Chen, P.P., Hathcock, J.J. & Taatjes, D.J. (2008) Hydroxychloroquine directly reduces the binding of antiphospholipid antibody-beta2-glycoprotein I complexes to phospholipid bilayers. *Blood*, **112**, 1687–1695.

Reber, G., Boehlen, F. & de Moerloose, P. (2008) Technical aspects in laboratory testing for antiphospholipid antibodies: is standardization an impossible dream? *Seminars in Thrombosis and Hemostasis*, **34**, 340–346.

Robertson, B. & Greaves, M. (2006) Antiphospholipid syndrome: an evolving story. *Blood Reviews*, **20**, 201–212.

Robertson, L., Wu, O., Langhorne, P., Twaddle, S., Clark, P., Lowe, G.D., Walker, I.D., Greaves, M., Brenkel, I., Regan, L. & Greer, I.A. (2006) Thrombophilia in pregnancy: a systematic review. *British Journal Haematology*, **132**, 171–196.

Rosborough, T.K., Jacobsen, J.M. & Shepherd, M. F. (2010) Factor X and factor II activity levels do not always agree in warfarin-treated lupus anticoagulant patients. *Blood Coagulation and Fibrinolysis*, **21**, 242–244.

Rosove, M.H. & Brewer, P.M. (1992) Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. *Annals of Internal Medicine*, **117**, 303–308.

Ruiz-Irastorza, G., Hunt, B.J. & Khamashta, M.A. (2007) A systematic review of secondary thromboprophylaxis in patients with antiphospholipid antibodies. *Arthritis and Rheumatism*, **57**, 1487–1495.

Sacre, K., Brihaye, B., Hyafil, F., Serfaty, J.M., Escoubet, B., Zennaro, M.C., Lidove, O., Laissy, J. P. & Papo, T. (2010) Asymptomatic myocardial ischemic disease in antiphospholipid syndrome: a controlled cardiac magnetic resonance imaging study. *Arthritis and Rheumatism*, **62**, 2093–2100.

Salmon, J.E. & Girardi, G. (2004) The role of complement in the antiphospholipid syndrome. *Current Directions in Autoimmunity*, **7**, 133–148.

Samarkos, M., Davies, K.A., Gordon, C. & Loizou, S. (2006) Clinical significance of IgA anticardiolipin and anti-beta2-GP1 antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. *Clinical Rheumatology*, **25**, 199–204.

Schjetlein, R., Sletnes, K.E. & Wisloff, F. (1993) A quantitative, semi-automated and computer-assisted test for lupus anticoagulant. *Thrombosis Research*, **69**, 239–250.

Schulman, S., Svenungsson, E. & Granqvist, S. (1998) Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. *American Journal of Medicine*, **104**, 332–338.

Shamonki, J.M., Salmon, J.E., Hyjek, E. & Baergen, R.N. (2007) Excessive complement activation is associated with placental injury in patients with antiphospholipid antibodies. *American Journal of Obstetrics and Gynecology*, **196**, 167 e161–165.

Simioni, P., Sanson, B., Prandoni, P., Tormene, D., Friederich, P., Girolami, B., Gavasso, S., Huisman, M., Buller, H., Wouter ten Cate, J., Girolami, A. & Prins, M. (1999) Incidence of venous thromboembolism in families with inherited thrombophilia. *Thrombosis and Haemostasis*, **81**, 198–202.

Sletnes, K.E., Gravem, K. & Wisloff, F. (1992) Preparation of plasma for the detection of lupus anticoagulants and antiphospholipid antibodies. *Thrombosis Research*, **66**, 43–53.

Stegnar, M., Bozic, B., Peternel, P., Kveder, T., Vene, N. & Rozman, B. (1991) Prevalence of antiphospholipid antibodies in deep vein thrombosis and their relationship to blood coagulation and fibrinolysis. *Thrombosis Research*, **63**, 433–443.

Stephenson, M.D., Ballem, P.J., Tsang, P., Purkiss, S., Ensworth, S., Houlihan, E. & Ensom, M.H. (2004) Treatment of antiphospholipid antibody syndrome (APS) in pregnancy: a randomized pilot trial comparing low molecular weight heparin to unfractionated heparin. *Journal of Obstetrics and Gynaecology Canada*, **26**, 729–734.

Swadzba, J., Iwaniec, T., Pulka, M., De Laat, B., De Groot, P.G. & Musial, J. (2011) Lupus anticoagulant: performance of the tests as recommended by the latest ISTH guidelines. *Journal of Thrombosis and Haemostasis*, **9**, 1776–1783.

Thom, J., Ivey, L. & Eikelboom, J. (2003) Normal plasma mixing studies in the laboratory diagnosis of lupus anticoagulant. *Journal of Thrombosis and Haemostasis*, **1**, 2689–2691.

Tietjen, G.E., Day, M., Norris, L., Aurora, S., Halvorsen, A., Schultz, L.R. & Levine, S.R. (1998) Role of anticardiolipin antibodies in young persons with migraine and transient focal neurologic events: a prospective study. *Neurology*, **50**, 1433–1440.

Tincani, A., Allegri, F., Balestrieri, G., Reber, G., Sanmarco, M., Meroni, P. & Boffa, M.C. (2004) Minimal requirements for antiphospholipid antibodies ELISAs proposed by the European Forum on antiphospholipid antibodies. *Thrombosis Research*, **114**, 553–558.

Topping, J., Quenby, S., Farquharson, R., Malia, R. & Greaves, M. (1999) Marked variation in antiphospholipid antibodies during pregnancy: relationships to pregnancy outcome. *Human Reproduction*, **14**, 224–228.

Tripodi, A., Chantarangkul, V., Clerici, M., Negri, B., Galli, M. & Mannucci, P.M. (2001) Laboratory control of oral anticoagulant treatment by the INR system in patients with the antiphospholipid syndrome and lupus anticoagulant. Results of a collaborative study involving nine commercial thromboplastins. *British Journal Haematology*, **115**, 672–678.

Tulppala, M., Marttunen, M., Soderstrom-Anttila, V., Foudila, T., Ailus, K., Palosuo, T. & Ylikorkala, O. (1997) Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2 production. *Human Reproduction*, **12**, 1567–1572.

Urbanus, R.T., Siegerink, B., Roest, M., Rosendaal, F.R., de Groot, P.G. & Algra, A. (2009) Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. *Lancet Neurology*, **8**, 998–1005.

Vila, P., Hernandez, M.C., Lopez-Fernandez, M.F. & Batlle, J. (1994) Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. *Thrombosis and Haemostasis*, **72**, 209–213.

Zoghlami-Rintelen, C., Vormittag, R., Sailer, T., Lehr, S., Quehenberger, P., Rumpold, H., Male, C. & Pabinger, I. (2005) The presence of IgG antibodies against beta2-glycoprotein I predicts the risk of thrombosis in patients with the lupus anticoagulant. *Journal of Thrombosis and Haemostasis*, **3**, 1160–1165.