Anticardiolipin antibodies and recurrent early pregnancy loss: a century of equivocal evidence

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In 1987, Nigel Harris cautioned against over-diagnosing the antiphospholipid syndrome (APS). In what was a rather prophetic editorial titled ‘The Syndrome of the Black Swan’, he suggested that while patients with APS do indeed exist, they are probably much more unusual than many medical professionals might like to believe. He expressed concern that the value of studying antiphospholipid antibodies (aPLs) as interesting non-organ specific autoantibodies, would become lost in a ‘sea of over-interpreted and over-reported laboratory and clinical findings’. It is our contention that 25 years later, this prediction has come to pass, particularly with respect to one type of aPL and its relation to a clinical event, namely anticardiolipin antibodies and early recurrent pregnancy loss. In this commentary, we trace the evolution of the current dogma and propose that reevaluation of available data from an alternative perspective results in quite a different understanding, the acceptance of which would necessitate not only a revision of the classification criteria for APS but also the subsequent revision of many diagnoses.

Key words: antiphospholipid syndrome / antiphospholipid antibodies / anticardiolipin antibodies / recurrent early pregnancy loss

Introduction

In 1906, Wasserman et al. reported the development of a complement fixation test for the detection of antibodies to syphilis. Pangborn (1941) identified cardiolipin as one of three lipoidal antigens (the others being lecithin and cholesterol) that comprise the Wasserman test. The name ‘cardiolipin’, originating from its initial extraction from the beef heart, has endured despite the fact that it is now known to be abundant in many tissues of many organisms (Schlake et al., 1993).

Both acute and chronic biological false-positive (BFP) tests for syphilis (reactive in a non-treponemal screen but non-reactive in a specific treponemal test) have been reported over the last century in individuals with neither history nor clinical symptoms of the infection, regardless of whether the Wasserman, the Venereal Disease Reference Laboratory (VDRL) or the rapid plasmin reagin test is employed (Kostant, 1956; Garner and Backhouse, 1972; Peter et al., 1979; Geusau et al., 2005). Acute BFP have been reported in a wide spectrum of patients including, but not limited to, blood donors, pregnant women, the recently vaccinated and patients with infectious disease other than syphilis, both bacterial and viral. Most patients with acute BFP seroconvert to negative, usually in less than 6 months.

In 1952a, b, Moore and Mohr reported a group of 51 patients with chronic BFP lasting longer than 6 months. Four had systemic lupus erythematosus (SLE), 23 had either history or clinical symptoms suggesting connective tissue disease and 14 were clinically healthy but had some laboratory abnormalities, most frequently an elevated erythrocyte sedimentation rate. These investigators were the first to identify this high prevalence in patients with SLE, and a BFP continues to be included in the serological classification criteria for the disease (Petri, 2009).

Since the first development of assays that enabled detection of antibodies specific for cardiolipin (Harris et al., 1983; Koike et al., 1984; Loizou et al., 1985), anticardiolipin antibodies (aCLs) have been identified in the same categories of patients who had been observed with BFP: healthy controls, the elderly, pregnant women, patients with bacterial and viral infections and in individuals post-vaccination, as well as those with SLE (Vaara et al., 1986; Manoussakis et al., 1987; Love and Santoro, 1990; Soloninka et al., 1995; Matthiesen et al., 1999; Cervera and Asherson, 2005; Perdan-Pirkmajer et al., 2012).

The lupus anticoagulant (LAC) and aCLs comprise a family of antiphospholipid antibodies (aPLs) frequently found in patients with SLE. Unlike aCL, the LAC is not detected by solid phase assays, but by prolonged clotting times in functional, phospholipid-dependent coagulation assays. This in vitro anticoagulant was first described by Conley and Hartmann (1952) but Bowie et al. (1963) noted that ~30% of patients with both SLE and the LAC actually presented with
thrombotic events. In October 1983, Hughes proposed a novel, pathological disorder distinct from SLE, the antiphospholipid syndrome (APS), within which the detection of LAC and aCLs play a crucial role.

Classification criteria for APS

APS has evolved over 29 years into a condition with a diverse spectrum of clinical associations but when first described, there was a limited set of classification criteria. These have been modified somewhat over the years (Table I), but still require evidence of both one or more specific, documented clinical events as well as the confirmed presence of one or more aPLs (Wilson et al., 1999; Miyakis et al., 2006). Clinical criteria comprise either an episode of vascular thrombosis and/or adverse pregnancy outcome including late pregnancy loss or recurrent early pregnancy loss (RPL). The laboratory criteria initially required the presence of the LAC and/or aCLs (Sapporo Criteria, 1999). This was subsequently modified to include the presence of anti-β2 glycoprotein IgG or IgM antibodies (anti-β2GPI; Sydney Criteria, 2006). In addition, the obstetric criteria were expanded to include placental insufficiency, and eclampsia and pre-eclampsia.

Etiology of aPLs

The mechanism(s) initiating the production of aPLs remain unknown (De Groot, 2011). However, like other autoimmune conditions, APS is considered to derive from a combination of environmental (infectious agents, trauma and drugs) and genetic factors (Merrill 2006; Sherer et al., 2007; Hughes, 2008; Meroni, 2008; Sene et al., 2008). aCLs have been detected in humans in association with a number of viral infections other than syphilis (Soloninka and Laskin, 1995; Gharavi and Pierangeli, 1998; Uthman and Gharavi, 2002; Gharavi et al., 2003). Further, studies with cytomegalovirus in mice indicated that a limited number of aPLs induced by various bacterial and viral products might be pathogenic in predisposed individuals (Gharavi et al., 2003) and aCLs have been termed either ‘infectious’ (usually the IgM isotype) or ‘autoimmune’ (Sene et al., 2008), although this distinction ‘may not be absolute’ (Asherson and Cervera, 2003). A review of animal models showed that mice immunized with various pathogenic agents developed clinical or laboratory evidence of APS-like disease (Harel et al., 2005), and it has been established that epitopes of β2GPI share similarities with common infectious pathogens. Anti-β2GPI titres were found to be particularly high after mice were injected with Haemophilus influenza and Neisseria gonorrhoea as well as tetanus toxoid (Inic-Kanada et al., 2009). In addition, Gharavi and Pierangeli (1998) reported that mice immunized with foreign β2GPI developed spinal cord infarction that resulted in intrauterine fetal death.

Although aCLs have been detected in association with many infections in humans, in only a few cases do these antibodies appear to have any pathogenic potential (Gharavi et al., 2001). In about one-third of cases of catastrophic APS, there is a clear association with a predisposing infection immediately prior to onset of the

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<tr>
<td><strong>Vascular thrombosis</strong></td>
<td>≥ 1 clinical episode of arterial, venous or small-vessel thrombosis in any tissue or organ; confirmed by imaging or Doppler studies or histopathology; no significant inflammation in the vessel wall</td>
<td>≥ 1 documented episode of arterial, venous or small vessel thrombosis—other than superficial venous thrombosis—in any tissue or organ; confirmed by objective validated criteria; no significant evidence of inflammation in the vessel wall</td>
</tr>
<tr>
<td><strong>Pregnancy mortality</strong></td>
<td>≥ 1 unexplained death of a morphologically normal fetus (documented by ultrasound or direct examination of fetus) at or beyond the 10th week of gestation or ≥ 3 unexplained consecutive spontaneous abortions before the 10th week of gestation with maternal anatomic or hormonal abnormalities, and paternal and maternal chromosomal abnormalities excluded</td>
<td>≥ 1 unexplained death of a morphologically normal fetus (documented by ultrasound or direct examination of the fetus) at or beyond the 10th week of gestation or ≥ 3 unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded</td>
</tr>
<tr>
<td><strong>Pregnancy morbidity</strong></td>
<td>≥ 1 premature birth of a morphologically normal neonate at or before the 34th week of gestation due to severe pre-eclampsia or eclampsia</td>
<td>≥ 1 premature birth of a morphologically normal neonate before the 34th week of gestation due to eclampsia or severe pre-eclampsia according to standard definitions, or recognized features of placental insufficiency</td>
</tr>
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**Table I** Comparison of the Sapporo (Wilson et al., 1999) and Sydney (Miyakis et al., 2006) criteria for the classification of the APS.
condition: at least in some cases therefore, aCL, produced perhaps as a result of molecular mimicry, may be immediately pathogenic (Ortega-Hernandez et al., 2009). A unifying proposal of 'second hits', including infectious agents, smoking, oral contraception, diet and lifestyle suggests that a predisposing factor is a necessary trigger for the production not only of cross-reacting aCL but also of an inflammatory response that potentiates thrombogenesis in genetically susceptible individuals (Sherer et al., 2007; Sene et al., 2008). The debate regarding the etiology of aPLs (and therefore the evolution of APS) continues.

**Laboratory evaluation of aCLs**

In 1985, the identification of aCLs by an ELISA was reported to be reliable, sensitive and specific (Loizou et al., 1985). By the early 1990s, however, it had become apparent that there were still issues to be resolved, from the correct selection of microtitre plate (for epitope presentation) to the identification and necessity of a plasma or serum cofactor (β2GPI) for aCLs binding to occur (Galli et al., 1990; Soloninka et al., 1991; Petri, 1994; Keil et al., 1995). In addition to methodological issues, standardization of aCL titres and variability of results both within and among laboratories became a focal point of aCL investigation (Clark et al., 2001).

Decades later, there are still controversies surrounding the significance and detection of aPLs, although there has been clarification of some points. IgA isotypes of both aCLs and anti-β2GPI were largely dismissed as insignificant with regard to APS (Bertolaccini et al., 2001), although anti-β2GPI IgA was recently implicated in thrombotic events in patients with SLE underlying APS (Mehrani and Petri, 2011). It is well established that co-factor (β2-GP1)-independent IgM aCLs are frequently detectable after a variety of viral infections (Bruce et al., 2000; Asherson and Cervera, 2003) so that their presence when screening potential patients for APS must be regarded with a degree of caution.

The Sydney classification criteria for APS expanded the necessary interval for a repeated positive aPLs from 6 to not less than 12 weeks to avoid false-positive aCL results attributable to transient infection, or to an erroneous prolongation of the LAC due to anticoagulation following an acute thrombotic event. It has been acknowledged that the decision to increase the time interval between tests was derived from expert opinion in the absence of validating studies (Miyakis et al., 2006). The updated criteria also included anti-β2GPI IgG and IgM, although their utility in classifying patients with APS, especially with respect to their exceedingly low prevalence among women with early RPL, continues to be questioned (Bruce et al., 2000; Guglielmine et al., 2001; Mehrani and Petri, 2011). However, the new criteria inexplicably dropped the requirement for aCLs to be measured using a β2GPI-dependent ELISA, thereby greatly reducing the specificity of the assay and enabling potential misclassification of patients with infection-related (β2GPI-independent) aCLs (Guglielmine et al., 2001).

With regard to assay standardization, most laboratories use commercial kits to detect aCLs and there is a continuing lack of consensus regarding: (i) the source, integrity and necessity of the β2GPI cofactor; (ii) the use of monoclonal antibodies as reference calibrators and (iii) the use of recommended cutpoints by manufacturers compared with those derived by each independent laboratory (Wong and Favaro, 2008; Pierangeli et al., 2011). Issues of standardization came to a head in 2008 with the publication of papers entitled ‘Is standardization an impossible dream?’ (Reber et al., 2008), ‘A quarter of a century in anticardiolipin testing and attempted standardization has led us to here, which is?’ (Pierangeli and Harris, 2008) and ‘A potpourri of problems, a compilation of possible solutions’ (Favaloro and Wong, 2008).

The absence of stringently acquired, reliable and reproducible laboratory data has resulted in the formulation of classification and treatment guidelines for APS based upon recommendations and personal preferences of the most influential investigators in the field (Miyakis et al., 2006; Hughes, 2008; Pierangeli et al., 2011). The guidelines have, as a result, been criticized for being ‘eminence rather than evidence based’ (Favaloro and Wong, 2008). Conflicting prevalence results and contradictory study outcomes have limited the clinical utility of aCL testing and undermined the validity of the laboratory criteria (Wong and Favaro, 2008; Pierangeli et al., 2011). Indeed Galli et al. (2008) proposed the exclusion of aCLs completely from the next update of APS criteria. At the 13th International Congress on aPLs, a recommendation was made to treat aPLs as markers or risk factors, rather than as a diagnostic focus (Pierangeli et al., 2011) reiterating concerns raised 24 years earlier that the LAC and aCLs should be regarded as markers rather than as etiological agents of pregnancy failure until more is understood about the pathological mechanisms involved (Kilpatrick, 1986).

**Obstetric APS: prevalence of aCLs and early RPL**

It is interesting to note that some investigators consider the association of aCLs and early RPL (defined in the APS classification criteria as ≥3 consecutive losses at <10 weeks’ gestation) as historically well established (Lockshin et al., 1985) perhaps because in the 1950s, a high prevalence of BFP was observed in patients with SLE, which carries an inherent increased risk of adverse obstetric events. Studies continue to report the presence of aPLs, whether aCLs or the LAC or both, in RPL but there is still no consensus, although it is generally accepted that fewer than one in five women with a history of early RPL but not SLE are positive for at least one aPL (Triplett and Harris, 1989; Gleicher, 1992; Laskin et al., 1997a; Brigham et al., 1999; American College of Obstetricians and Gynecologists, 2001; Empson et al., 2005; Opatrny et al., 2006; Devreese and Hoylaerts, 2009; Pengo and Ruffatti, 2010; Jaslow et al., 2010; Urbanus and De Groot, 2011).

The prevalence and the association of aCLs have been investigated from a number of sampling perspectives including assessments of the general obstetric population, patients with unexplained spontaneous losses and those with SLE. In a 1989 study of prevalence and biologic significance of aCLs in a large general obstetric population, Lockwood et al. found that aCL IgG or IgM were rarely present (16/737) and were correlated only weakly with adverse outcomes. In 1990, Love et al. evaluated the prevalence of aCLs in SLE and non-SLE patients and found no association with a history of fetal loss in non-SLE patients and a suggested, but not firmly supported, association in patients with SLE.

In 1991, Infante-Rivard et al. selected 331 women with one spontaneous abortion or fetal death and 993 controls, measured aCL prevalence and concluded there was no justification for considering...
aCL a risk factor for fetal loss in the absence of a prior adverse obstetric event. This finding elicited considerable debate, as evidenced by no fewer than five letters to the editor, each of which criticized either the inclusion criteria, determination of cutpoints for antibody titres, inadequately characterized and inappropriately grouped fetal losses or technical issues with the assay used (Branch et al., 1992; Birdsall and Pattison, 1992; Gharavi and Asherson, 1992; Harris et al., 1992; Lockshin and Sammaritano, 1992). Almost 15 years later, it was reported that maternal thrombophilias (acquired or inherited) including aCLs were not associated with pregnancy loss prior to 10 weeks’ gestation following an investigation of 491 women with a history of adverse obstetric events (Rojé et al., 2004). In addition, Mehrani and Petri (2011) reported no association between aCLs and pregnancy loss in 569 women with SLE who had ever been pregnant, 241 of whom had a history of pregnancy loss.

A meta-analysis in 2006 concluded that the magnitude of association between aPLs and RPL in women without SLE varied and depended upon which aPL was measured (Opatrny et al., 2006). Even among studies of sufficient design stringency to qualify for inclusion in the meta-analysis (25 of an initially retrieved 128 published studies), the authors noted the usual suspects: poor standardization of assays, inclusion of women with other causes of RPL, inconsistent selection of controls, variability of aPLs or isotype tested and varying definitions of RPL (differing numbers and gestational ages of losses). No association was found with anti-β2GPI while there was an association between LAC and RPL. The association with aCL IgG and IgM appeared variable and dependent upon titre and gestational age. The authors reiterated that prospective human data on the relationship between aPLs and RPL were still lacking and that the pathophysiology of their role in uteroplacental insufficiency remains incompletely understood.

It may be an epidemiologic truism that an association between a common occurrence and a rare marker cannot be demonstrated (Lockshin and Sammaritano, 1992). However, many of the studies described above attempted to identify an increased prevalence of aCLs within highly selected patient samples and even within that bias were unable to establish a definitive association between aCLs and RPL.

In addition to this inconsistency, there is also the issue of identification of etiology of early pregnancy loss with or without aCL. As Macklon et al. (2002) noted, >30% of clinically recognized pregnancies end before the completion of the first trimester, and the majority of those losses are caused by fetal chromosomal abnormalities. Due to technical, logistic and financial obstacles, cytogenetic studies of the products of conception are only completed successfully in a minority of cases. Taken together, the absence of chromosomal investigation of the aborted fetus and the incrementally higher risk of loss in any pregnancy following a loss (Brigham et al., 1999) make it increasingly problematic to attribute an aPL-related causality to any early pregnancy loss.

### Obstetric APS: pathologic evidence

Initially, it was hypothesized that aPL-mediated placental thrombosis and impaired placental blood supply were responsible for the adverse obstetric events in APS (De Wolf et al., 1982; Hughes, 1983; Khamashta et al., 1989; Out et al., 1991; Triplett, 1992; Magid et al., 1996; Pierangeli and Harris, 1996; Rand, 1998; Kandiah et al., 1998; Field et al., 1999; Ieko et al., 1999). Early proposals regarding the activation of the coagulation cascade included various mechanisms (decreased prostacyclin production, increased thromboxane production, decreased protein C activation and annexin V displacement) either in the maternal circulation or at the feto-maternal interface. However, aPL-mediated-placental pathology is currently understood to be inconsistent at best (Franco et al., 2011; Beeksm et al., 2012). Furthermore, as Abou-Nasser et al. (2011) noted in a recent meta-analysis, studies evaluating this association have frequently been inadequately powered to draw any conclusions.

Over the past 30 years, many potential mechanisms have been postulated to identify a causative link. Using an animal model of thrombosis, Pierangeli and Harris (1996) showed that human polyclonal and monoclonal aCLs, derived from a patient with APS, reversibly enhanced thrombus formation in mice, providing insight into a potential relationship between aCLs and fetal loss (De Wolf et al., 1982). While some studies supported the pathogenicity of aPLs in animal models, for example an increase in fetal resorption after intrauterine injection of human aCLs into pregnant rats (Radway-Bright et al., 1999; Halperin et al., 2002), there were also reports of inconsistent pregnancy outcomes after the same treatment in mice. Silver et al. (1998) found that although there was greater murine fetal resorption after injection with affinity purified human IgG from aCL-positive patients compared with aCL-negative IgG or saline, there was marked variability among pregnancy outcomes in the aCL-positive animals that was not attributable to the aCL activity in the original serum sample or in the purified extract.

Histopathological examination of the decidua from women with clinical symptoms of APS but no aPL showed the same level of necrosis, inflammation and vascular thrombosis as women with aPLs and clinical symptoms (Van Horn et al., 2004). This finding indicated that aPLs were neither necessary nor sufficient for the adverse obstetric events observed. In 2004, Salmon and Girardi proposed complement activation as a central mechanism in aPL-induced pregnancy loss and placental infarction based upon observations after passive transfer of human aCLs into pregnant mice. They acknowledged that the cause of tissue damage due to aCLs was likely multifactorial, but proposed complement activation as an absolute requirement for the most serious phenotypic outcomes. Weiler (2008) noted that placental infarction was conspicuously absent from the placentas of aPL-infused mice, and that anticoagulation therapy with heparinoids or the selective thrombin inhibitor, hirudin, did not prevent fetal loss in mice. Indeed, Girardi et al. (2004) had earlier reported that heparin was effective in preventing aPL-induced fetal loss in mice because of its inhibition of complement activity, not because of its anticoagulant activity.

Hills et al. (2006) using first trimester human villous and extravillous trophoblast cell lines, demonstrated heparin’s cytoprotective effect as it conferred resistance to apoptosis triggered by a variety of pathological stimuli, including the proinflammatory, anti-prothrombotic molecule and tumor necrosis factor α (TNF-α). It is noteworthy that aPLs have been reported to provoke a rapid complement-dependent increase in TNF-α both systemically and at the maternal–fetal interface (Smadja et al., 2010). It is also possible that aPL-mediated complement
 Activation disrupts normal angiogenic factors leading to a decrease in placental vascularization (Singh, 2001). Alternatively, aCL binding to antigens on endothelial and trophoblast cell surfaces might be causing, among other direct damage, defective placentation.

Kornberg et al. (1994) observed the induction of a tissue factor-like procoagulant activity after stimulation of mononuclear cell lines with a monoclonal aCL. Amengual et al. (1998) confirmed that tissue factor levels were elevated in patients with APS and suggested this as a pathogenic etiology for thrombosis in patients with aPLs. Recently, Redecha et al. (2008) reported that neutrophils from aPL-treated mice up-regulated the expression of protease-activated receptor 2 and tissue factor, which led to neutrophil activation, trophoblast injury and fetal death. However, Girardi (2011) reported tissue factor-mediated trophoblast injury and embryo damage present in both aPL-associated and antibody-independent mouse models of RPL. In addition, a study using a transgenic mouse model of ischemic stroke designed to monitor the adverse effect of aCL on post-ischemic outcome found that aPL treatment unexpectedly resulted in decreased infarct volumes (Frauenknecht et al., 2011). A study of 108 documented placental infarctions concluded that maternal thrombophilias have at most a weak association, as almost 80% had histologic evidence of non-infarct uteroplacental vascular pathology and fewer than 4% of the women had aCL positivity (Franco et al., 2011). This vast, and in some cases conflicting, array of putative aPL-mediated mechanisms illustrates either the obvious ambiguity or the extraordinary complexity of the potential actions of aPLs.

Despite this variety of proposed mechanisms, the accumulated pathologic data, comprising in vivo animal and in vitro human studies, still do not indicate a direct causal relationship between aCLs and early pregnancy loss in humans (Urbanus and De Groot, 2011). A recent review proposed the possibility of pathogenic mechanisms and laboratory markers related to aPL-related pregnancy loss ‘which have yet to be elucidated’ (Alijotas-Reig and Vilardebell-Tarres, 2010). While there is emerging evidence that aCLs may be pathogenic among genetically susceptible individuals as a result of adverse intra-vascular events or in the presence of coincident risk factors (Giron-Gonzalez et al., 2004) or ‘second hits’, there remains the distinct possibility that aCLs may be only a complicating epiphenomenon rather than a pathogenic mechanism of early RPL (Laskin and Soloninka, 1988; Rand, 1998).

### Obstetric APS: clinical trials

Because the adverse obstetric events associated with aPLs were originally observed in patients with SLE, initial therapeutic interventions to improve the pregnancy outcome intuitively involved immunosuppressive treatment. Four trials evaluated the efficacy of prednisone and aspirin (ASA) for RPL with aPLs (Lockshin et al., 1989; Cowchock et al., 1992; Harger et al., 1995; Laskin et al., 1997a, b). Investigators concluded that prednisone did not confer additional benefit over ASA alone and that it may have been associated with the higher rate of premature births seen in each trial. As a result, the treatment with prednisone for aPL-positive early RPL uncomplicated by SLE is no longer considered appropriate (Empson et al., 2005).

Over the past 15 years, a number of randomized controlled trials for patients with RPL with aPLs have evaluated the efficacy of either unfractionated (UFH) or low-molecular-weight heparin (LMWH) in combination with ASA treatment (Kutteh, 1996; Rai et al., 1997; Farquharson et al., 2002; Franklin and Kutteh, 2002; Noble et al., 2005; Laskin et al., 2009; Table III). Each trial determined its own aPL inclusion criteria, including cutpoints for aCLs, and each center measured the LAC using different methodologies. These design inconsistencies might be expected to have resulted in differential outcomes. However, regardless of the variable laboratory criteria, the live birth rates, whether in the UFH or LMWH treatment arms, were similar, ranging from 71.1 to 84%. The only significant differences among trial outcomes were found in the ASA-only treatment arms (Table III): live birth rates varied from a low of 42.2% to a high of 80.0%. Interestingly, both the lowest and the highest birth rates with ASA only treatment occurred in trials with the lowest cutpoints for aCL positivity (Rai et al., 1997, Pattison et al., 2000), and the second lowest birth rate occurred in a trial that excluded LAC positive patients altogether (Kutteh, 1996).

Intuitively, given that the LAC is currently considered the aPL most probably associated with adverse obstetric outcomes, the exclusion by Kutteh et al. of LAC-positive women from their trial should have

### Table II Comparison of aPL inclusion criteria and live birth rates among studies investigating the efficacy of UFH and LMWH for early recurrent pregnancy loss.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>n</th>
<th>IgG aCL (GPL)</th>
<th>aCL IgM (MPL)</th>
<th>Positive LAC</th>
<th>Intervention</th>
<th>% Live births</th>
</tr>
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<tbody>
<tr>
<td>Kutteh (1996)</td>
<td>25</td>
<td>≥27</td>
<td>≥27</td>
<td>Excluded</td>
<td>ASA + sc UFH</td>
<td>80.0</td>
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<tr>
<td>Rai et al. (1997)</td>
<td>45</td>
<td>&gt;5</td>
<td>&gt;3</td>
<td>RVVT</td>
<td>ASA + sc UFH</td>
<td>71.1</td>
</tr>
<tr>
<td>Cowchock et al. (1992)</td>
<td>26</td>
<td>&gt;30</td>
<td>&gt;11</td>
<td>DRVVT or APPT</td>
<td>ASA + sc UFH</td>
<td>73.1</td>
</tr>
<tr>
<td>Franklin and Kutteh, (2002)*</td>
<td>25</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>DRVVT</td>
<td>ASA + LMWH</td>
<td>76.0</td>
</tr>
<tr>
<td>Farquharson et al. (2002)</td>
<td>51</td>
<td>&gt;9</td>
<td>&gt;5</td>
<td>DRVVT</td>
<td>ASA + sc LMWH</td>
<td>78.4</td>
</tr>
<tr>
<td>Noble et al., (2005)*</td>
<td>25</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>DRVVT</td>
<td>ASA + LMWH</td>
<td>80.0</td>
</tr>
<tr>
<td>Laskin et al. (2009)</td>
<td>22</td>
<td>&gt;15</td>
<td>&gt;25</td>
<td>DRVVT, PTT-LA, DilPT, KCT</td>
<td>ASA + sc LMWH</td>
<td>77.3</td>
</tr>
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</table>

*Studies that also included patients with antiphospholipid serine antibodies.

**DRVVT, dilute Russell’s viper venom time; PTT-LA, lupus anticoagulant-sensitive partial thromboplastin time; DilPT, dilute prothrombin time; KCT, kaolin clotting time; ASA, aspirin; UFH, unfractionated heparin; LMWH, low-molecular-weight heparin; sc, subcutaneous. Reproduced with permission from Laskin et al. (2009).**
resulted in an improved birthrate. Similarly, the inclusion of women by Rai et al. with very low positive levels of aCLs should also have resulted in an improved birthrate in the ASA-only treatment arms. These two trials with the lowest ASA-only birth rates, however, were among the earliest interventional results reported for aPL-associated RPL, and were also among the very few that have reported a significant benefit for heparin and ASA over ASA treatment alone. Despite the heterogeneity of their study populations, lack of fulfillment of APS criteria and the absence of confirmation of results from subsequent trials by others (Farquharson et al., 2002; Franklin and Kutteh, 2002; Noble et al., 2005; Laskin et al., 2009), their findings, usually cited together, helped establish the current standard of care that continues to recommend the use of heparin and ASA for women with early RPL and aPLs. A Cochrane review concluded that further large trials of heparin (both UFH and LMW) combined with ASA and ASA alone are needed to reduce clinically important uncertainty about the benefits and harms, and to assess their efficacy and interchangeability (Empson et al., 2005). Nevertheless, evidence to the contrary notwithstanding, this review still recommended UFH and ASA for pregnancy loss with aPLs, while cautioning that this was based on only two small trials (Kutteh, 1996; Rai et al., 1997) one of which, they noted, lacked adequate allocation concealment.

There is a growing body of evidence that does not support the use of UFH or LMWH plus ASA over ASA alone in this population. Gates et al. (2002) concluded after reviewing the literature, that for women with aPLs, early RPL and no prior history of thrombosis, there is insufficient evidence to base recommendations for thrombophrophylaxis in pregnancy. The lack of consistent and predictable outcomes in trials ostensibly evaluating the same RPL population may be due to lack of assay standardization described above in addition to considerable variation in patient selection: some trials included women with both early and late losses, some specifically excluded women with a history of thrombosis and some accepted women with aCL IgM antibodies but excluded those with the LAC (Gleichner, 1992; Branch, 1998; Branch et al., 2010). This latter point, the heterogeneity of aPL positivity among women included in these trials, may be the pivotal consideration for the absence of clarity and consensus with regard to treatment outcome.

Further clouding interpretation of results of clinical trials of heparin treatment for RPL in patients with aPLs, Franco et al. (2011) reiterated Hills’ earlier proposition (2006) that heparin may be effective in preventing gestational morbidity and mortality but not because of its anticoagulant properties. They remarked that clinical trials of heparin for aPL-associated pregnancy morbidity to date have had an underlying assumption that anticoagulant therapy was required to counteract maternal thrombophilia thereby rendering the placenta invulnerable to thrombosis. Unfortunately, there has been an absence of placental pathology studies included in these clinical trials to sustain this hypothesis. Franco et al. proposed that in studies where heparin has apparently had a positive effect on pregnancy outcome, it is as a result of its diverse actions affecting trophoblast differentiation and vascular function independent of the presence (or absence) of aPLs.

It is becoming apparent that the LAC, and not aCLs, may be the aPL associated with late adverse obstetric outcome (Firkin et al., 1980). In a prospective observational study of stringently defined aPL-positive pregnancies both with and without SLE, the LAC was the strongest predictor of serious pregnancy complications (Lockshin et al., 2012). High titres of anti-β2GPI antibodies and aCLs (≥40 GPL units, therefore fulfilling APS criteria) were not associated with adverse obstetric events including, but not limited to, early pregnancy loss. Those findings are in concert with observations of Mehrani and Petri (2011) that neither aCLs nor anti-β2GPI antibodies were associated with pregnancy loss in a sample of aPL-positive patients with SLE and a history of pregnancy loss. In addition, long-term follow-up studies have shown that the vast majority of women with RPL and aPLs will not have any subsequent thrombotic sequelae or develop autoimmune disease even as long as 20 years after initial presentation (Silver et al. 1994; Ercan et al., 2001; Tincani et al., 2002; Clark et al., 2005, 2009; Quenby et al., 2005; Lim et al., 2006; Ercan et al., 2007; Cervera et al., 2009; Shoenfeld et al., 2009).

### Conclusion

In conclusion, therefore, it is apparent that the classification of APS, its etiology, pathogenesis and prognosis all remain somewhat ill-defined. Thousands of papers have been published in this area and yet many aspects describing this syndrome seem to defy clarification. Numerous reports either support or dispute earlier findings regarding the presence or absence of aPL-associated cause and clinical effect as well as treatment efficacy but there is no conclusive or consistent evidence.

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**Table III** Comparison of live birth rates among ASA-only treated arms of clinical trials investigating the efficacy of different therapeutic regimens for early recurrent pregnancy loss.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>n</th>
<th>Positive aCL IgG (GPL)</th>
<th>Positive aCL IgM (MPL)</th>
<th>Positive LAC</th>
<th>% Live births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowchock et al. (1992)</td>
<td>19</td>
<td>&gt;30</td>
<td>&gt;11</td>
<td>DRVVT or APPT</td>
<td>68.4</td>
</tr>
<tr>
<td>Kutteh (1996)</td>
<td>25</td>
<td>≥27</td>
<td>≥27</td>
<td>Excluded</td>
<td>44.0</td>
</tr>
<tr>
<td>Rai et al. (1997)</td>
<td>45</td>
<td>&gt;5</td>
<td>&gt;5</td>
<td>RVVT</td>
<td>42.2</td>
</tr>
<tr>
<td>Pattison et al. (2000)</td>
<td>20</td>
<td>≥5</td>
<td>≥5</td>
<td>aPTT, DRVVT, KCT</td>
<td>80.0</td>
</tr>
<tr>
<td>Farquharson et al. (2002)</td>
<td>47</td>
<td>&gt;9</td>
<td>&gt;5</td>
<td>DRVT</td>
<td>72.3</td>
</tr>
<tr>
<td>Laskin et al. (2009)</td>
<td>21</td>
<td>&gt;15</td>
<td>&gt;25</td>
<td>DRVVT, PTT-LA, DIPT, KCT</td>
<td>76.2</td>
</tr>
</tbody>
</table>

ASA, aspirin; DRVVT, dilute Russell’s viper venom time; PTTLA, lupus anticoagulant sensitive partial thromboplastin time; DIPT, dilute prothrombin time. Reproduced with permission from Laskin et al. (2009).
Many patients with a long history of thrombotic events or RPL, in the absence of comorbidities like SLE, are either negative for or have fluctuating levels of aPLs (Clark et al., 2005). It was reported that a history of atrial fibrillation, congestive heart failure and valvular heart disease, for example, were found to be associated with significantly higher levels of aCL IgG but it was observed that the increases in immunoreactivity were a function, in a dose-dependent manner, of the number of cerebrovascular risk factors also present (Tanne et al., 1999). The authors therefore cautioned against over-diagnosis with APS and the consequent changes in management among patients with multiple risk factors. In 1992, Ginsberg et al. found no association of aCLs with ischemic stroke but did find an association with moderate to high levels in patients with venous thromboembolism. In contrast, Runchey et al. (2002) concluded, after completing a study of almost 1000 patients, that there was no association between venous thromboembolism and aCLs. A cautious approach is therefore required, not only in terms of therapy, but also in terms of classification within APS (Clark et al., 2001, 2002).

Intuitively, given the classification criteria for APS, one would assume at least a consistent correlation between the presence of aPLs and clinical manifestations of the syndrome. However, this is not the case (Clark et al., 2005, 2007). The majority of women with early RPL are negative for aPLs as are the majority of patients presenting with deep vein thrombosis and stroke (Devreeze and Hoylaerts, 2009). Hughes (2008) proposed that the ratio of ‘1 in 5 is a useful guide’: 1 in 5 patients with RPL have aPLs and 1 in 5 patients with deep vein thrombosis have aPLs. The corollary to this proposition is that four out of five patients with RPL do not have aPLs; neither do four out of five patients with deep vein thrombosis. Conversely, a minority of women with uneventful pregnancies has been shown to be positive for aPLs and in a study following up asymptomatic, persistently aPL-positive patients with no clinical manifestations of APS, investigators found a low overall annual incidence of thrombosis and no benefit accrued by prophylactic treatment with low dose ASA (Opatrny et al., 2006). It is of interest to note that treatment does not differ for patients with recurrent thromboembolism regardless of the presence or absence of aPLs (Lim et al., 2006; James, 2009; Tuthill and Khamashta, 2009). It is the recurrent event itself, and not the presence of aPLs, that determines the treatment regimen.

‘Seronegative APS’, first proposed in 1997 (Miret et al., 1997), describes patients with a typical idiopathic clinical picture suggestive of APS, but without evidence of circulating aPL. The concept of seronegative disease is not new. Clinically active, serologically quiescent lupus has been described for >30 years (Gladman et al., 1979, 2003). However, unlike APS, classification with SLE comprises fulfillment of at least four criteria among a number of clinical and laboratory elements, not a specific requirement for a specific autoantibody (Tan et al., 1982). Seronegative APS is therefore both a puzzling and intriguing entity: classification with this syndrome has a definitive requirement for a positive aPL on at least two occasions. In the absence of aPLs, is it not the idiopathic clinical event just the clinical event? Perhaps by introducing this seronegative variant, the investigators inadvertently undermined the very nature of the syndrome by declaring that a heretofore-essential classification element is not necessarily required.

Classification with APS carries the burden of significant long-term health and financial implications (i) because of the influence of classification on subsequent disease management; (ii) because of its designation by insurance companies as a potentially uninsurable condition (Assurant Health, Genworth Financial and Mutual of Omaha underwriting guidelines) and (iii) because once classified with this syndrome, there are no mechanisms for reversal of the classification. It follows, therefore, that classification criteria should be as specific and sensitive as possible.

Our contemplation of the literature in conjunction with our own experience over the past 25 years leads us to the conclusion that there is a need for redefinition of APS (Clark, 2010). Withdrawal of aCL-associated early RPL from the classification criteria appears justified by the inconsistent prevalence of aCLs in this population and the increasing body of evidence that this clinical manifestation of APS is distinct from late loss or early delivery with placental infarction (Bramham et al., 2010). It is difficult to rationalize the continued inclusion of a group of aCL-positive women, whose only clinical manifestation of APS is early RPL, under the same umbrella as those with adverse late gestational morbidity and mortality, catastrophic venous or arterial thrombosis and the potential requirement for lifelong anticoagulation. In 1998, Branch, echoing Harris’ earlier concerns regarding the potential for over-diagnosis of APS (1985), cautioned that, ‘Though most authorities require the presence of either lupus anticoagulant or medium-to-high titer IgG aCL to make a diagnosis of antiphospholipid syndrome, in some series no more than half of the study patients had lupus anticoagulant and as many as 20% had only IgM aCL. It is very unlikely that patients with such disparate clinical and laboratory findings have the same autoimmune syndrome’.

It has been just over a century since the first use of the Wasserman test and subsequent identification of the BFP. In 1910, at a weekly meeting of the Berlin Medical Society, several physicians expressed the necessity for caution in accepting results of the Wasserman test and Wasserman himself, who was present at the meeting, protested against ascribing conflicting or erroneous results to the methodology. He suggested that any interlaboratory inconsistencies were the direct result of either inexpert technique or the use of reagents not specifically recommended by him. An editorial, commenting on the discourse, reiterated the importance of practitioners becoming informed as to the reliability of the tests they order, and the absolute necessity of interpreting those laboratory results in the context of their own clinical observations (Jones, 1910). This admonition is as appropriate today as it was a century ago.

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