Laboratory Diagnosis of Lupus Anticoagulants

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Circulating anticoagulants or inhibitors of clotting factors are defined as endogenously produced substances that interfere with various in vitro tests of coagulation.\(^\text{1}\) Anticoagulants are usually immunoglobulins, although other endogenous materials, such as heparin or fibrinogen split products, may inhibit in vivo or in vitro coagulation (Table 1). Specific inhibitors are immunoglobulins with epitope specificity for a single coagulation protein. These inhibitors may be neutralizing or non-neutralizing.\(^\text{2,3}\) The most common specific inhibitors are auto or alloantibodies to Factor VIII.\(^\text{4}\) It is imperative to identify correctly Factor VIII inhibitors, since they are associated with significant clinical bleeding.\(^\text{1}\) Nonspecific inhibitors, such as lupus anticoagulants (LA) are not directed at any single coagulation protein and they are generally not associated with bleeding.\(^\text{5}\)

Conley and Hartmann\(^\text{6}\) first reported an association between circulating anticoagulants and systemic lupus erythematosus (SLE).\(^\text{6}\) Their first case report emphasized a correlation with bleeding; however, subsequent studies have shown that these patients generally do not have a bleeding tendency attributable to the coagulation inhibitor. The term “lupus anticoagulant”\(^\text{7}\) was suggested in 1972 by Feinstein and Rapaport.\(^\text{7}\) It is, however, an unfortunate misnomer, since the majority of patients do not have SLE and, in the absence of other hemostatic abnormalities, the patients do not bleed.

Since patients with LA generally lack hemorrhagic symptoms, clinicians and laboratories initially regarded LA as a nuisance. However, laboratories were required to investigate the causes of abnormal activated partial thromboplastin times (APTT). Often the evaluation of the abnormal APTT involved multiple factor assays and time-consuming mixing studies yielded inconclusive or confusing results. Thus, some reagent manufacturers designed their reagents to be insensitive to the presence of LA.

Paradoxically, LA was subsequently found to be associated with both arterial and venous thrombosis as well as recurrent pregnancy loss.\(^\text{3,8,9}\) Consequently, laboratories are now receiving requests to evaluate patients for the presence of LA. This review will focus on the characterization, clinical associations, and laboratory diagnosis of LA.

**CHARACTERIZATION OF LUPUS ANTICOAGULANTS**

LAs are immunoglobulins (usually IgG, occasionally IgM, IgA, or a mixture) that interfere with in vitro phospholipid-dependent coagulation tests, such as prothrombin time (PT), APTT, and dilute Russell’s viper venom time (dRVVT).\(^\text{1,7}\) These antibodies do not specifically inhibit any of the coagulation factors; rather, they appear to be directed at phospholipid epitopes.\(^\text{5}\) Early case reports of LA often noted an association with biologic false-positive tests for syphilis or abnormal flocculation tests of liver function. Laurell and Nilsson\(^\text{10}\) found cardiolipin used in the VDRL test system would partially adsorb LA from plasma. Thiagarajan et al\(^\text{11}\) subsequently isolated an IgM monoclonal LA from a patient with Waldenström’s macroglobulinemia. This monoclonal LA reacted with anionic phospholipids but did not prevent in vitro platelet-dependent prothrombinase activity. However, when phospholipid micelles were substituted for platelets, in vitro coagulation reactions were inhibited. These observations further established the phospholipid specificity of LA and also provided insight into the lack of clinical bleeding.

Although it is now clear the LAs react with phospholipids, the precise epitope or epitopes with which LAs react are poorly understood. Harris et al\(^\text{12}\) have demonstrated a close relationship between LA and antiphospholipid antibodies.
TABLE 1. Classification of Circulating Anticoagulants*

<table>
<thead>
<tr>
<th>Specific</th>
<th>Neutralizing</th>
<th>Non-neutralizing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V</td>
<td>Factor VIII</td>
<td></td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Factor IX</td>
<td>von Willebrand’s factor</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Factor X</td>
<td></td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Factor XIII</td>
<td></td>
</tr>
<tr>
<td>von Willebrand’s factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonspecific</td>
<td>Lupus cogenetic anticoagulants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paraproteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrin split products†</td>
<td></td>
</tr>
<tr>
<td>Global</td>
<td>Heparin-like activity</td>
<td></td>
</tr>
</tbody>
</table>

* From Triplett and Brandt.77 Reprinted with permission. † As originally defined: Fibrinogen degradation products (FDP).

cardioplin antibodies (ACA). However, other investigators have not found the same degree of concordance.13,14 Some monoclonal antibodies with LA activity cross-react with DNA and cardiolipin.15 These findings suggest that DNA and phospholipids share a common epitope that reacts with LA. The regularly spaced phosphodiester groups found in both DNA and phospholipids were initially proposed as the putative epitope. However, the inability of LA to bind to neutral phospholipids, such as phosphatidylethanolamine (PC), phosphatidylethanolamine (PE) would suggest another epitope.16

Janoff and Rauch17 have approached the question of LA specificity using monoclonal hybridoma LA antibodies derived from patients with SLE. These antibodies were selected based on their ability to prolong a dilute APTT. Initially, the monoclonal LAs were evaluated using various phospholipids to inhibit the LA in the APTT test system.18 These experiments required incubation of the LA with phospholipids prior to adding the mixture to the APTT assay. They found PE inhibited the LA activity, whereas PC and phosphatidylinositol (PI) did not. Thus, these results were divergent from the studies of Thiagarajan et al.11 Further studies by Rauch et al18 suggested that LAs react with lipids that assume a hexagonal configuration at 37° C.

Hexagonal phospholipid configurations can occur in vivo with membrane damage.19,20 Thus, LA may represent antibodies produced in response to an altered membrane phospholipid configuration secondary to injury (such as viral, drugs, tumor necrosis factor).

**CLINICAL CORRELATIONS**

LA may be identified in a variety of clinical conditions, including autoimmune disorders, as a result of certain medications, following infections, and malignancies (Table 2).

In most general laboratory settings, LAs are detected serendipitously as a result of routine laboratory screening. The reported prevalence in unscreened general populations or in preselected diseases such as SLE has varied widely,21 This variability is due to patient selection, as well as test sensitivity and specificity. Also, the diligence of the technologists performing the assay is critical in identification of LA. LAs are often transient and, considering their association with infectious diseases, probably occur at one time or another in a significant percentage of apparently normal persons of all ages.

The incidence of LA in autoimmune diseases has been most thoroughly evaluated in SLE. Early studies suggested an incidence of approximately 10%;22,23 however, more recent studies have reported values of 21 to 65%.24 Patient selection, disease activity, criteria for the laboratory diagnosis, and treatment all contribute to these differences.

Drug-induced LAs have been reported with hydralazine, chlorpromazine, quinidine, and procainamide; these drugs have all been implicated in drug-induced SLE-like syndromes. Other drugs associated with LA include various antibiotics and phenytoin.25 In four large series of patients with LA, drug-associated LA was identified in 6% (14 of 219),26 34% (34 of 100), 4% (4 of 100),27 and 15% (5 of 33).28 Drug-related LAs have been most frequently reported with chlorpromazine and procainamide.29,30 In a series of 47 patients receiving

**TABLE 2. Clinical Conditions Associated with Lupus Anticoagulants**

<table>
<thead>
<tr>
<th>Autoimmune diseases</th>
<th>Systemic lupus erythematosus</th>
<th>Rheumatoid arthritis</th>
<th>Others, including &quot;overlap&quot; syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug exposure</td>
<td>Chlorpromazine</td>
<td>Procainamide</td>
<td>Hydralazine</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
<td>Antibiotics</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>Infections</td>
<td>Bacterial</td>
<td>Protozoan (Pneumocystis carinii)</td>
<td>Viral (HIV-1)</td>
</tr>
<tr>
<td></td>
<td>Fungal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>Hairy cell leukemia</td>
<td>Malignant lymphoma</td>
<td>Waldenström’s macroglobulinemia</td>
</tr>
<tr>
<td>Miscellaneious disorders</td>
<td>Epithelial malignancies</td>
<td>No underlying diseases</td>
<td></td>
</tr>
</tbody>
</table>

* From Triplett and Brandt.77 Reprinted with permission.
chlorpromazine for longer than 2½ years, 41 developed LA. Most drug-related LAs have been IgM. 29,31 Although early reports indicated no thrombotic predisposition in patients with drug-related LAs, more recent studies have found an incidence similar to other LA patients. 13

Most transient LAs are seen in the setting of infectious diseases. 25,32 Often, LAs are detected in children as the result of a preoperative coagulation screen prior to tonsillectomy and adenoidectomy. LAs have also been identified in patients with the acquired immune deficiency syndrome (AIDS) (Table 3). 33-36

The associated infections may be protozoan, viral, fungal, or bacterial. With successful treatment of the infectious disease, the LA will usually disappear. 37 ACA have also been found in patients with AIDS. 38 Familial cases of LA have been reported. 39,40 In a significant percentage of LA cases, no underlying disease is identified.

**CLINICAL COMPLICATIONS ASSOCIATED WITH LUPUS ANTICOAGULANTS**

The identification of LA or antiphospholipid antibodies (APA) has received increased attention due to the association between these antibodies and a variety of clinical complications.

The paradoxical occurrence of thromboembolic events in patients with in vitro hypocoagulable plasma remains an intriguing and important, although as yet, unexplained observation. LA/APA have also been associated with spontaneous abortion, fetal death, and a variety of other obstetric complications 1-3 (Table 4). LA/APA have also been implicated as a cause of infertility. 41

**Thromboembolic Complications**

Bowie et al 8 were the first to report an increased risk of thrombosis in patients with LA. The cumulative literature suggests approximately 30% of patients with LA will have at least one thrombotic event. 32 Unlike hereditary deficiencies of antithrombin III, protein C, and protein S, patients with LA have both arterial and venous thrombosis. 2,43,44

The arterial thrombi most often involve the cerebral vessels with clinical presentation as strokes, transient ischemic attacks, and amaurosis fugax. 45 Histologically, the involved arteries and veins are characterized by bland thrombosis with no evidence of vessel wall inflammation.

Whether LAs are involved in the pathogenesis of the thrombotic episodes or merely represent an epiphenomenon is currently unresolved. The frequency of thrombosis seen in association with LA is perhaps skewed due to selection biases. The presence of LA in patients with SLE appears to identify a subset of patients with a three- to four-fold increased incidence of thrombosis. Often, the LA may appear years before the first thrombotic episode. In order to resolve the role of LA in thrombosis, there is a need for a well-designed prospective study to evaluate a broad spectrum of patients with LA.

A variety of potential pathophysiologic mechanisms have been proposed to explain the association of LA and thrombosis. Carreras et al 46 first suggested LA may prevent the mobilization of arachidonic acid from endothelial cell membranes resulting in decreased prostacyclin (PGI₂) production. 46 Since PGI₂ is a potent inhibitor of platelet aggregation, decreased amounts of PGI₂ may potentially contribute to thrombosis. These original studies were carried out using heterologous systems (human plasma and rat aortic endothelium). Subsequent studies using homologous in vitro experiments have yielded conflicting results. 37,48

Other hypotheses have focused on abnormal fibrinolysis, platelet activation, and abnormal antithrombin III activity. 49-51 Perhaps the most promising investigations have focused on the interaction of LA and endothelial cells. Endothelial cells may express both anticoagulant and procoagulant properties. Under physiologic conditions, the anticoagulant properties are predominant.

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**TABLE 3. Lupus Anticoagulant and AIDS**

<table>
<thead>
<tr>
<th>Author</th>
<th>No. Patients</th>
<th>LA</th>
<th>Immunoglobulin Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Freer et al</td>
<td>25</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td>Bloom et al</td>
<td>38</td>
<td>28</td>
<td>—</td>
</tr>
<tr>
<td>Cohen et al</td>
<td>50</td>
<td>10</td>
<td>6/7 IgM</td>
</tr>
<tr>
<td>Gold Zalusky</td>
<td>52</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td></td>
<td>77 (47%)</td>
</tr>
</tbody>
</table>

* LA was often transient.

**TABLE 4. Obstetric Complications Associated with Lupus Anticoagulants**

<table>
<thead>
<tr>
<th>Complication</th>
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</thead>
<tbody>
<tr>
<td>Maternal</td>
</tr>
<tr>
<td>Arterial and venous thromboembolism</td>
</tr>
<tr>
<td>Accelerated preeclampsia</td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
</tr>
<tr>
<td>Postpartum syndrome</td>
</tr>
<tr>
<td>Chorea gravidarum</td>
</tr>
<tr>
<td>Fetal</td>
</tr>
<tr>
<td>Intrauterine fetal death</td>
</tr>
<tr>
<td>Intrauterine fetal growth retardation</td>
</tr>
<tr>
<td>Neonatal thrombosis</td>
</tr>
<tr>
<td>Premature labor and delivery</td>
</tr>
<tr>
<td>Recurrent spontaneous abortion</td>
</tr>
</tbody>
</table>
Several groups have now demonstrated the ability of LA to interfere with the activation of protein C by the thrombin-thrombomodulin complex. The activation of protein C is a calcium ion, phospholipid-dependent reaction that occurs in vivo on the endothelial surface. Activated protein C is an important regulatory protein that preferentially degrades activated forms of the cofactors V and VIII. Decreased protein C activity is associated with clinical venous and rarely arterial thrombosis. Additional studies are needed to evaluate this potential mechanism of LA-induced thrombosis further.

A variety of substances, including tumor necrosis factor, interleukin 1, and endotoxin, may induce endothelial cell production of tissue factor (TF). TF is a lipoprotein that is the cofactor for the extrinsic pathway of Factor X activation. Recently, Rustin and colleagues studied sera from patients with SLE in an experiment designed to evaluate the expression of cultured endothelial cell procoagulant activity. Using a two-stage coagulation assay, SLE sera caused an increase in procoagulant activity. The expression of TF by endothelial cells required de novo synthesis of protein. Perhaps the increased TF found in these experiments may be explained by antibody binding to endothelial cells, with resulting membrane perturbation and subsequent TF production.

Obstetric Complications

Nilsson and colleagues were the first to describe an association between recurrent abortions and LA. Soulier and Boiff subsequently described the triad of LA, recurrent abortions, and thrombosis. Since these first reports, several additional retrospective studies have confirmed the clinical syndrome of repeated abortions with LA or ACA. Most commonly, the women will have a history of first trimester abortion or second trimester fetal death. The initial reports stressed the presence of placental infarction as the cause of fetal loss. However, in many cases the degree of placental impairment due to the infarction appears to be insufficient to account for fetal death. Decreased PGI₂ production by fetal and maternal tissues has also been suggested as an explanation for fetal loss. The pattern of decreased PGI₂ production in women with LA is similar to that seen in preeclampsia. Studies that have evaluated LA in women with histories of recurrent abortions have found an incidence varying from 5.2 to 48%.

Thrombocytopenia

Thrombocytopenia is also associated with LA. In patients with SLE, thrombocytopenia is seen in the LA-positive group with an incidence of 50 to 60%. In contrast, 25% of the SLE/LA-negative patients have a complicating thrombocytopenia. Other patient groups with LA have varying degrees of thrombocytopenia.

Cutaneous Manifestations

Cutaneous manifestations associated with LA range from livedo reticularis to widespread cutaneous necrosis. Livedo reticularis is a clinical finding closely linked to ACA and LA. LAs have also been described in patients with Degos’ disease and Behçet’s syndrome. Both of these conditions are associated with thrombotic complications.

Bleeding Complications Associated with Lupus Anticoagulants

Although the presence of LA alone is not associated with bleeding, a number of concomitant abnormalities may predispose the patient to hemorrhage (Table 5). Occasionally, patients with LA may have a specific coagulation inhibitor, such as Factor VIII antibody. Antibodies to Factor VIII may be particularly difficult to identify in a patient with a LA. This situation may arise in patients with autoimmune diseases (SLE), with hemophilia A, or complicating AIDS with a LA. Factors V and XI inhibitors may be very difficult to separate from a LA. Therefore specific factor assays are necessary in patients with an apparent LA and associated clinical hemorrhage. Acquired prothrombin deficiency is also associated with LA.

LABORATORY IDENTIFICATION OF LUPUS ANTICOAGULANTS

Although the Working Party on Acquired Inhibitors of Coagulation of the International Committee on Thrombosis and Haemostasis proposed a set of criteria to diagnose LA, these criteria have not been widely accepted. The lack of acceptance is no doubt due to the reliance on one- and two-stage factor assays. Factor assays are expensive and two-stage factor assays are not widely used in routine laboratories. Triplett and Brandt have re-

<table>
<thead>
<tr>
<th>TABLE 5. Potential Causes of Bleeding in Patients with Lupus Anticoagulants</th>
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<tbody>
<tr>
<td>Specific factor inhibitors</td>
</tr>
<tr>
<td>Factor VIII inhibitors</td>
</tr>
<tr>
<td>Factor II inhibitors</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>Uremia</td>
</tr>
<tr>
<td>Concomitant drug administration (aspirin)</td>
</tr>
</tbody>
</table>
cently proposed minimal criteria that incorporate the concept of phospholipid specificity of LA. These criteria are: (1) an abnormality of a phospholipid-dependent coagulation test (in most instances this will be an unexplained abnormal APTT); (2) demonstration that the abnormality is due to an inhibitor; and (3) proof that the inhibitor is directed at phospholipids.

The laboratory approach to the diagnosis of LA may be staged according to the criteria just mentioned. Screening procedures will usually identify an unexplained prolongation of the APTT or PT. In many instances the findings of an abnormal APTT are the result of a routine coagulation screen. Once an abnormal APTT is found, the next step is mixing studies to establish the presence of an inhibitor. The final step in the evaluation requires confirmatory procedures to identify phospholipid specificity of the inhibitor.

Although historically the identification of LA was usually a serendipitous finding, there has been a dramatic change within the last 5 years. This change is secondary to the identification of LA-associated clinical findings of abortion and recurrent thrombosis and the concept of the phospholipid antibody syndrome, which broadens the laboratory diagnosis to include immunologic procedures to identify APA. Consequently, many laboratories are now being asked: ‘Does this patient have a lupus anticoagulant?’ Thus, the laboratory must develop a systematic approach to this question rather than the historical reactive approach to an unexplained coagulation result.

Preanalytic Variables

Perhaps the most important step in the evaluation of patient samples for LA involves careful specimen collection and processing. The centrifugation step is critical, since the ability of coagulation assays to detect LA is generally inversely proportional to the number of platelets in the platelet-poor plasma (PPP). Careful preparation of PPP will also improve the stability of frozen samples; PPP with significant residual platelets will have considerable shortening of a prolonged APTT on freeze-thawing. Millipore-filtered plasma and the commercially available serum-plasma separators will yield excellent PPP.

Screening Procedures

A variety of tests have been used to screen for the presence of LA. In the vast majority of laboratories the APTT is used for this purpose. The popularity of the APTT is due to its commercial availability, ease of automation, and simplicity. Although there is wide variation in sensitivity to LA, when a sensitive and responsive reagent is used, the APTT performs as well if not better than other test systems (such as dRVVT, kaolin clotting time (KCT), and plasma clot time (PCT)). The differing sensitivity of APTT reagents is due to a number of variables, including amount, composition, and configuration of phospholipid, surface activator, and other properties, such as buffering capacity. Also the marked heterogeneity of LA from patient to patient should be emphasized. No single test system or reagent will identify all patients with LA.

The KCT is commonly used as a screening procedure for LA in Europe and Australia. The KCT is performed on PPP with no added phospholipids. Exner et al. have described several patterns of KCT when plasma samples containing LA were mixed with varying amounts of normal plasma. The major disadvantage of the KCT is an inability to be automated. Based on limited studies, the KCT appears to be the most sensitive test for detecting LA in the setting of pregnancy.

Identification of Inhibitor Activity

Once an abnormal screening procedure is identified, the next step in the laboratory evaluation is to rule out the presence of occult heparin contamination. The most common cause of an unexplained prolonged APTT in a hospitalized patient is heparin (Fig. 1).

The presence of heparin may be easily identified by using a thrombin time or reptilase time. Heparin may also be neutralized using a cation exchange resin or protamine sulfate. However, the addition of substances to

FIG. 1. Approach to prolonged APTT.
neutralize heparin will interfere with further coagulation testing.

Once heparin is ruled out, the next step is a mixing study. Most laboratories utilize mixing studies of 1 part patient plasma to 1 part normal plasma.\textsuperscript{86} If there is a failure to correct the prolonged APTT, the diagnosis of a circulating inhibitor is established.

Mixing studies are often very difficult to interpret. In some instances, a 1:1 mix of LA and normal plasma may actually correct to within the normal range. Brandt et al\textsuperscript{82} observed this phenomenon on 16% of LA plasmas. Although this occurred more often in the case of borderline APTTs, it was also observed with very long patient baseline APTTs. Conventional wisdom has relied on the identification of LA by an immediate effect (that is, LAs do not require incubation to demonstrate maximal anticoagulant activity). In contrast, Factor VIII inhibitors often exhibit a time dependence. In most instances, the Factor VIII inhibitors with low titer will have such a pattern. Triplett et al\textsuperscript{21} were the first to stress the incidence of a time-dependent pattern with LA. In a subsequent study, Brandt et al\textsuperscript{82} found that 10 to 15% of LA showed a clear-cut time-dependent inhibition and an additional 15 to 20% showed a borderline time-dependent prolongation. Lazarchick et al\textsuperscript{83} also found a 15% incidence of time dependency LA. Subsequently, Silverman et al\textsuperscript{87} also found a frequency of 40% time-dependent LA (10 of 25). Clyne et al\textsuperscript{88} also found a frequency of 40% (21 of 52) in a retrospective study of LA.\textsuperscript{88} These three recent studies emphasize the necessity of time-dependent mixing studies in the evaluation of prolonged APTTs. In the Clyne study, 8% of cases would have been missed if only immediate mixing studies had been performed.

The evaluation of a minimally prolonged APTT (for example, less than 7 sec above the upper limit of the normal range) is frequently misleading because it is often difficult to distinguish "correction" from "dilution" of inhibitor effect. When evaluating such plasmas, a mixture of 4 parts patient to 1 part normal is more sensitive to the presence of an inhibitor.\textsuperscript{89}

Occasionally, mixing studies will yield a paradoxical accentuation of the patient’s prolonged APTT or other screening tests. An unidentified factor that is present in normal plasma and responsible for this effect has been termed the "lupus cofactor."\textsuperscript{90} This cofactor effect is most commonly seen when a 4:1 patient to normal mix is used. Also low concentrations of phospholipids will accentuate the "cofactor."\textsuperscript{91} Jude et al\textsuperscript{27} also noted an apparent sensitivity of the lupus cofactor effect to the choice of activator in the APTT reagent. Reagents with ellagic acid apparently do not identify the "cofactor effect."

In cases in which the prolonged screening test is only minimally outside the upper limit of the normal range, mixing studies may be inconclusive. In these cases, the use of plasma-agarose gel is very helpful.\textsuperscript{92}

### Confirmatory Procedures

Following the identification of an inhibitor, it must be characterized. LA must be differentiated from specific inhibitors that are associated with clinical bleeding. A variety of tests have been proposed to identify LA. These tests have relied on two basic approaches: Either increasing the amount or altering the configuration of the phospholipid in the test system to bypass or neutralize the LA or decreasing the phospholipid concentration to accentuate the LA effect (Table 6).

The tissue thromboplastin inhibition (TTI) test is the most commonly used test to confirm the diagnosis of LA.\textsuperscript{93} Unfortunately, the TTI test is not specific for LA; consequently, reliance on the TTI test as a diagnostic procedure is unfounded.\textsuperscript{80,94} False-positive results may be seen with specific factor inhibitors (such as Factor VIII inhibitors), factor deficiencies, and with anticoagulant therapy. One study suggested that 40% of normal persons will have an abnormal TTI test.\textsuperscript{94} Recently, Liu and colleagues\textsuperscript{95} reported on a modified dilute thromboplastin inhibition test.\textsuperscript{95} They used varying dilutions of human brain thromboplastin and a platelet neutralization procedure to increase the specificity of the TTI. Using this approach, their system identified all LA with no false-positive results.

The dRVVT is also used as a confirmatory procedure for LA.\textsuperscript{96} The dRVVT appears to be sensitive to the presence of LA and in several studies has been utilized as both a screening procedure as well as a confirmatory test. In some patients who are receiving immunosuppressive therapy, the APTT will return to normal, but the dRVVT will remain prolonged.\textsuperscript{96}

The KCT has been a useful test to identify and characterize LA.\textsuperscript{85,97} The KCT may be used to evaluate patients further with minimally prolonged APTTs or in patients with clinical findings of the phospholipid anti-

### Table 6. Confirmatory Procedures to Identify LA

<table>
<thead>
<tr>
<th>Test procedures</th>
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<tbody>
<tr>
<td>Tissue thromboplastin inhibition (TTI)\textsuperscript{95}</td>
</tr>
<tr>
<td>Dilute Russell viper venom time\textsuperscript{86}</td>
</tr>
<tr>
<td>Kaolin clotting time\textsuperscript{85}</td>
</tr>
<tr>
<td>Dilute phospholipid APTT\textsuperscript{104}</td>
</tr>
<tr>
<td>High phospholipid APTT\textsuperscript{102}</td>
</tr>
<tr>
<td>Rabbit brain neutralization\textsuperscript{94}</td>
</tr>
<tr>
<td>Phosphatidylserine liposome APTT\textsuperscript{84}</td>
</tr>
<tr>
<td>Hexagonal phospholipid neutralization\textsuperscript{18}</td>
</tr>
</tbody>
</table>
body syndrome in whom an APTT is normal. The KCT may be used on patient plasma alone or on mixtures of patient and normal plasma. Several different patterns have been described when mixtures of patient and normal plasmas are used.\textsuperscript{85,91} An additional advantage of the KCT is its reliability in identifying LA in patients receiving warfarin. Disadvantages of the KCT are the requirement for filtered plasma and the fact that it is a manual procedure.

The platelet neutralization procedure (PNP) is based on the ability of platelets to bypass or significantly correct in vitro coagulation abnormalities.\textsuperscript{86} In early studies, platelets activated by adenosine diphosphate, collagen, or inophore (A23187) were utilized.\textsuperscript{98} Subsequently, the use of washed freeze-thawed platelets proved to be effective in significantly correcting the inhibitor effect of LA. In a recent study by Kornberg et al\textsuperscript{99} the PNP was compared with the TTI test and KCT index. The PNP was positive in 98% of the 48 patients, whereas the TTI test was positive in 79% and the KCT index in 77%. Thus, the PNP appears to be the most sensitive of the confirmatory tests.\textsuperscript{94} Lesperance et al\textsuperscript{100} also found the PNP to be the most sensitive confirmatory test in their study. The properties of the platelet preparations or phospholipids used in neutralization or bypass procedures are of great importance. In an evaluation of 20 patients with LA, Dayton et al\textsuperscript{101} used freeze-thawed platelets, a commercial source of platelet extract (platelet extract reagent, BioData Corp.) and a concentrated solution of standard APTT reagent. The PNP using freeze-thawed platelets was positive in 20 of 20 patients, whereas the platelet extract reagent was positive in 9 of 20 and the concentrated APTT reagent in 5 of 20.

A variety of additional tests have been described, including the high phospholipid APTT of Clyne et al,\textsuperscript{102} the rabbit brain neutralization of Rosove et al,\textsuperscript{94} and the phosphatidylserine (PS)-liposome APTT of Kelsey et al.\textsuperscript{84} As mentioned earlier, the hexagonal phospholipid neutralization procedure of Rauch et al\textsuperscript{18} may prove to be the most specific test for LA. In a recent report, Rauch and Janoff\textsuperscript{103} evaluated ten LA plasma samples from patients with SLE, six plasmas containing specific anticoagulants, and five factor-deficient plasmas. They used a preincubation step using hexagonal phase PE and a dilute APTT assay. The hexagonal PE correctly identified all LA plasmas and was negative with samples containing specific inhibitors or factor deficiencies.

When factor assays are performed on LA plasma, the factors of the proximal intrinsic pathway are most commonly affected.\textsuperscript{105} Typically, Factors XII and XI are more affected than Factors VIII and IX. With dilution of the patient’s plasma, the apparent factor activity will increase. This lack of parallelism of the factor assay curves is typical of plasma containing an inhibitor. Factor assays performed using synthetic substrates are usually not affected by the presence of LA.

### Immunologic Assays: Phospholipid Antibody Test

In 1983, Harris et al\textsuperscript{67} developed a solid phase radioimmunoassay for ACA.\textsuperscript{67} This assay is 200 to 400 times more sensitive than the standard VDRL. Subsequently, it was found that other negatively charged phospholipids, such as PS, PI, and phosphatidic acid (PA) could also be used as antigen in this assay enzyme-linked immunosorbent assay [ELISA] or radioimmunoassay [RIA].\textsuperscript{13} Thus, a more generic term “antiphospholipid antibodies” was introduced. These antibodies have been associated with the same clinical complications seen in association with LA. Harris\textsuperscript{106} proposed the concept of an “antiphospholipid antibody syndrome,” which would include patients with APA whether or not a LA is present (Table 7).

In most early studies, patients with LA had APA.\textsuperscript{107,108} However, there is considerable heterogeneity with approximately 30% of LA patients having no associated APA.\textsuperscript{13,100}

Commercial sources of ELISA assays for APA are now available. These kits use either cardiolipin or a mixture of negatively charged phospholipids as antigen. An APA assay should be a part of the laboratory evaluation of any patient with clinical findings suggestive of the phospholipid antibody syndrome.

### TREATMENT

The presence of LA or APA in an asymptomatic patient is not an indication for treatment. Although these

### TABLE 7. Proposed Criteria for Antiphospholipid Antibody Syndrome*

<table>
<thead>
<tr>
<th>Clinical†</th>
<th>Laboratory‡</th>
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</thead>
<tbody>
<tr>
<td>Venous thrombosis</td>
<td>IgG anticardiolipin antibody (&gt;10 GPL units)§</td>
</tr>
<tr>
<td>Arterial thrombosis</td>
<td>Positive lupus anticoagulant test§</td>
</tr>
<tr>
<td>Recurrent fetal loss</td>
<td>IgM anticardiolipin antibody (&gt;10 MPL units)§ and positive LA test</td>
</tr>
</tbody>
</table>

* From Harris.\textsuperscript{106} Reprinted with permission.
† Patients with the APA syndrome should have at least one clinical and one laboratory finding during the disease. The APA test should be positive on at least two occasions more than 8 weeks apart.
‡ GLP and MPL refer to IgG and IgM phospholipid antibodies. The units refer to the standards proposed by Harris et al\textsuperscript{15} at the second International Workshop on phospholipid antibodies (April 4, 1986).
§ The lupus anticoagulant should be confirmed by correction of the prolonged clotting studies with freeze-thawed platelets (platelet neutralization procedure).
patients may have an increased risk of thrombosis, available laboratory evaluation is not able to subdivide patients with APA/LA into high- or low-risk groups. There is some evidence to suggest that patients with IgG APA of high titer represent a high-risk group. However, other studies have not confirmed these findings. The presence of APA/LA in patients who are undergoing major surgery or prolonged confinement to bed would warrant the use of low-dose heparin prophylaxis (5000 U twice daily subcutaneously).

Patients with LA or APA who present with arterial or venous thrombosis are treated initially with heparin and then oral anticoagulation. Although corticosteroids may lead to normalization of the APTT, they are not an effective means of preventing recurrent thrombosis in patients with APA/LA.

Lubbe et al. were the first to treat young women with LA and a history of spontaneous abortion with prednisone (40 to 60 mg/day) and low-dose aspirin (75 mg/day). Although corticosteroids are not without complications, this regimen has proved effective in many cases. Other forms of therapy that have been described include intravenous immunoglobulin, heparin, plasma exchange, and immunosuppressive agents.

In order to resolve the questions surrounding the clinical management of pregnancies in women with APA, the Fetal Loss Subcommittee of the Kingston Antiphospholipid Study Group has proposed a multicenter clinical trial to evaluate therapeutic management of this patient group.

REFERENCES


