

LUPUS ANTICOAGULANTS: A PROTEAN LABORATORY PHENOMENON

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Introduction

Antiphospholipid-protein antibodies (APA) are a family of autoimmune and alloimmune immunoglobulins (IgG, IgM, IgA or mixtures) that recognize protein phospholipid complexes^(1,2) (Table 1). These antibodies have been identified utilizing a variety of different laboratory test systems including solid phase radioimmunoassays, ELISA assays and coagulation tests. Much of the literature describing APA has focused on the phospholipid (PL) used in the various assay systems. The concept of plasma proteins playing an important part in these assays has emerged over the last six years beginning with the description of b2 Glycoprotein I (Apolipoprotein H) as a cofactor for the anticardiolipin antibody (ACA) test.^(3,4)

The APA family includes reagin (the antibody detected in serologic tests for syphilis), ACA, and lupus anticoagulants (LA). LA and ACA are closely-related antibodies. In patients with autoimmune disease, these two antibodies occur with a concordance of approximately 70%. Early studies suggested LA and ACA were the same antibodies; however, more recent studies have clearly demonstrated they are different antibodies.⁽⁵⁾ Therefore, it is important for laboratories to evaluate patients with findings suggestive of the antiphospholipid antibody syndrome (APS) for *both* of these antibodies.⁽⁶⁾

This review will focus on LA. In the early 1950s, Mueller et al. and Conley/Hartmann were the first to describe a peculiar circulating anticoagulant (synonym: inhibitor) in patients with systemic lupus erythematosus (SLE).^(7,8) Conley and Hartmann suggested patients with this inhibitor were at risk for bleeding; however, subsequent case reports and patient series consistently emphasized the incongruity between laboratory test results and clinical findings. With rare exceptions, patients with LA *do not* bleed. Ironically, a significant number of patients (approximately 30%) were found to have either venous or arterial thromboembolic events.⁽⁹⁾ LA are immunoglobulin(s) (IgG, IgM, IgA or mixtures) which interfere with one or more of the *in vitro* PL-dependent tests of coagulation, for example, activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute Russell viper venom time (dRVVT), and dilute prothrombin time (dPT).^(1,2) LA may be seen in diverse clinical settings (Table 1).

Table 1. Classification of Antiphospholipid Protein Antibodies

- I) Autoimmune
 - a) Primary
 - Do not fulfill criteria for systemic lupus erythematosus
 - b) Secondary
 - Systemic lupus erythematosus

- Other connective tissue diseases
 - c) Drug induced
- II) Alloimmune
 - a) Infections
 - Viral
 - Bacterial
 - Protozoal
 - Fungal
 - b) Malignancies
 - Hairy cell leukemia
 - Lymphoproliferative
 - Epithelial

Clinical Presentations

Serendipitous Detection

LA may present in any patient population. Often, they are detected as a part of routine preoperative screening. In this setting, the patients are most frequently detected by an unexplained prolonged APTT. Pediatric patients are often screened with routine coagulation tests prior to tonsillectomy and adenoidectomy. In this patient group, an abnormal APTT is not uncommon. In many cases, the abnormal APTT results in cancellation of surgery and an extensive coagulation evaluation. This patient group exemplifies the frustration associated with the diagnosis of LA. Most of these patients are receiving antibiotics because of recurrent middle ear infections and tonsillitis. The vast majority of these patients (prolonged APTT) ultimately prove to have alloantibodies manifesting as LA. Alloantibodies (LA or ACA) are typically transient and are not associated with the clinical complications seen with autoimmune APA. The pediatric patient group is frustrating not only to the surgeons but also the laboratory. Because of the prolonged APTT, factor assays are often performed. In many cases, factor activity is decreased on initial dilution but shows a progressive apparent increase with further serial dilution.⁽¹⁰⁾ Factor assays most commonly affected are factors XI, XII and to a lesser extent factors VIII and IX. The results of the factor assays coupled with the abnormal APTT creates a dilemma for the surgeon, the patient and the patient's family, and the laboratory. Frequently the patient is discharged from the hospital and requested to return after six to eight weeks for further evaluation. Upon re-evaluation of these patients, not infrequently the APTT is found to be normal. This type of transient prolongation of the APTT is typical of alloimmune infection-related LA. In today's environment of managed care, the routine use of preoperative coagulation testing is discouraged. As a result, the serendipitous detection of LA has decreased in all patient groups.

The Bleeding Patient

Detection of LA in the bleeding patient is uncommon. There are three clinical settings in which LA may be found in association with bleeding: (a) LA and

thrombocytopenia in the patient with APS, (b) LA and hypoprothrombinemia, and (c) LA associated with hemophilia A or B.

LA and concordant thrombocytopenia are relatively common in patients with APS.⁽¹¹⁾ The thrombocytopenia appears to be immunologic in origin. Consequently, one must interpret the platelet count and its relationship to bleeding in the same context seen with patients with other autoimmune thrombocytopenias.⁽¹²⁾ Platelet counts in the 50,000 to 60,000/mm³ range are infrequently associated with bleeding. It is important in this patient group to be aware of other underlying clinical situations which may compromise platelet function (e.g., use of nonsteroidal antiinflammatory agents, uremia, gastrointestinal ulcers, etc.).

In the majority of patients with LA, human prothrombin is the protein component of the antigenic protein phospholipid complex.^(13,14) When large series of patients with LA have been screened using crossed immunoelectrophoresis, approximately 75% of patients are found to have circulating antigen antibody complexes (prothrombin-LA).⁽¹³⁻¹⁵⁾ These antiprothrombin antibodies have been identified as nonneutralizing (i.e., do not react with the functional site[s]). In most patients, the antiprothrombin antibody activity is of low affinity. These patients do not demonstrate hypoprothrombinemia but do have evidence of circulating antigen antibody complexes.⁽¹⁵⁾ In patients with the high affinity APAs, the antigen antibody complexes are cleared from plasma, resulting in acquired selective deficiency of prothrombin.^(16,17) Patients with selective prothrombin deficiency are at risk for bleeding. Often, they present with mucosal bleeding (epistaxis or gastrointestinal bleeding).

The occurrence of LA in the setting of hemophilia A or B is often confusing and, in many cases, difficult to diagnose. LA that appears in this setting are usually alloimmune resulting from the exposure of the patient to a multiple viral infectious agents as a result of replacement therapy. LA are frequently recognized in HIV positive patients. As part of comprehensive care of patients with hemophilia A, they are screened for the presence of inhibitors routinely and preoperatively. The detection of an inhibitor in the hemophilia patient is extremely important. If the inhibitor is a specific antibody directed to factor VIII or factor IX, the subsequent management of the patient becomes much more difficult. It is critical to identify the nature of an inhibitor detected in a hemophilia patient. Historically, laboratories have relied on the concept of time-dependent inhibitory activity as being specific for factor VIII inhibitors and immediate inhibitory activity suggesting LA. Recent studies indicate approximately one-third of LA will show time-dependence of inhibitory activity.⁽¹⁸⁾ Thus, it is inappropriate to rely upon time dependency as a means of differentiating a factor VIII inhibitor from LA. Factor IX inhibitors present an even more difficult problem since they are typically immediate inhibitors.

Recurrent Spontaneous Abortion/Fetal Loss

Obstetricians are very aware of the association of LA and ACA with a variety of obstetrical complications.^(1,2,19) The most frequently encountered are recurrent fetal loss (often late first trimester/second trimester), fetal growth retardation, early onset preeclampsia, and prematurity. Women with a history of recurrent fetal loss are often evaluated for the presence of LA/ACA. If these tests are positive, they are treated during

subsequent pregnancies with a variety of agents including heparin, aspirin, prednisone, and intravenous immunoglobulin.⁽²⁰⁾ Because of the physiologic changes in coagulation associated with pregnancy, the diagnosis of LA in a pregnant woman is often difficult.⁽²¹⁾ Routine screening studies such as the APTT or dPT may be normal even though there is an underlying LA. In this setting, it is appropriate to screen the patient with more sensitive tests such as the KCT.

Thrombotic Patients

Bowie and colleagues were the first to describe an association between LA and thrombotic events.⁽⁹⁾ In their original series of patients, they also recognized the clinical finding of livedo reticularis as being associated with LA. With the description of APS in the early 1980s, testing for APA has become a part of the laboratory evaluation of patients with thromboembolic disorders.⁽²²⁾ The thrombotic events seen in association with LA may involve virtually any anatomic site (arteries, veins, and capillaries). On the venous side of the circulation, deep vein thrombosis (DVT) is most commonly encountered. The most common arterial site is the cerebral circulation. For many years, the question of whether APA was the cause of thromboembolic events was widely debated. Recent evidence utilizing prospective studies, case-control studies, and cross-sectional studies all suggest these antibodies are causative.⁽²³⁾ The mechanism is most likely multifactorial. Venous thromboembolic events in most cases appear to be secondary to inhibition of the activated protein C system while arterial events represent abnormalities of the platelet/endothelial balance.^(2,24-27) A problem frequently encountered in patients with thrombosis and an associated LA is the question of heparin monitoring. Worldwide, the APTT is the most common test used to monitor unfractionated heparin. When patients present with a prolonged baseline APTT secondary to LA and a concordant thrombosis, clinicians are often in a quandary. The question posed to the laboratory is: "How shall I monitor this patient with a prolonged baseline APTT?" There are three basic approaches, all of which are successful: (a) monitor with an antifactor Xa assay system (synthetic substrate based), (b) monitor with a thrombin time, or (c) utilize an APTT reagent that is insensitive to LA. The choice as to which of these approaches is utilized depends upon the supporting laboratory.

Unusual Presentations of LA

On rare occasions, LA may prove to be a very elusive diagnosis. In some instances, the initial laboratory studies suggest an isolated factor deficiency (e.g., factor IX or factor XI). The resolution of these cases requires careful laboratory analysis including performing factor assays utilizing synthetic substrates. Also, it may be necessary to evaluate these patients for antigenic levels of the factors in question.

Another unusual presentation of LA is the selected prolongation of the prothrombin time. This is rarely encountered. However, with the introduction of more "sensitive" prothrombin reagents, it may become more frequent. Recombinant thromboplastins appear more sensitive to LA than the animal thromboplastins (rabbit brain/rabbit brain and lung).

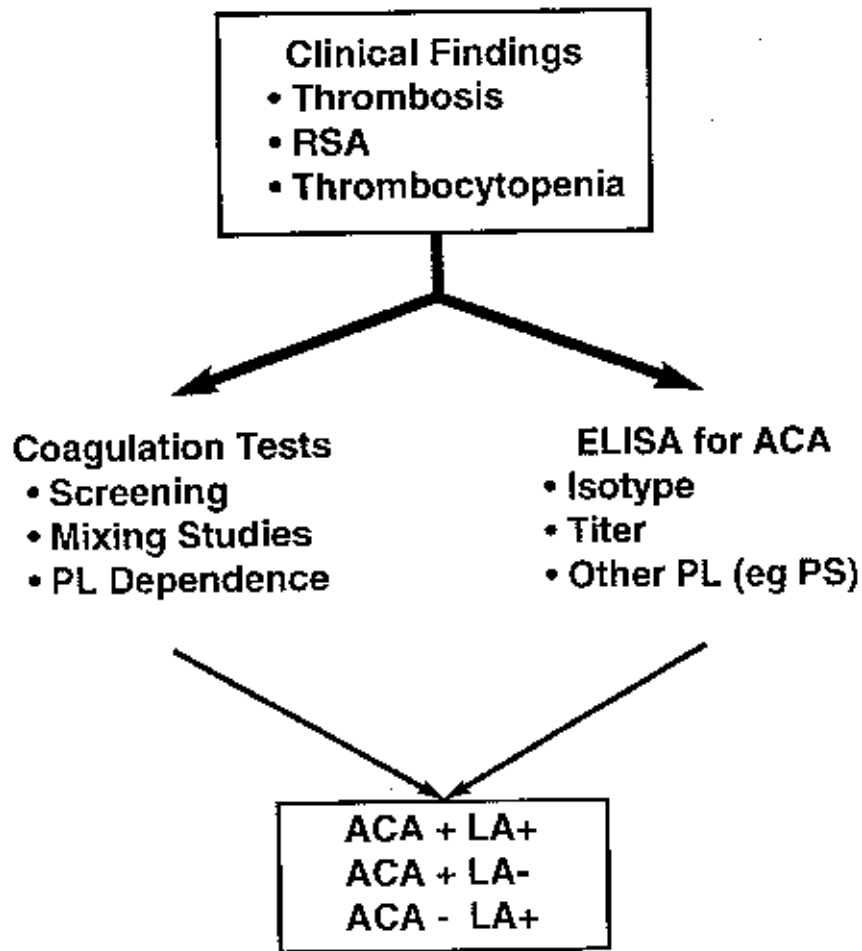
The protean nature of LA is exemplified by the wide range of clinical presentations outlined above. Virtually any clinician may encounter patients with LA. The

laboratory physician must be vigilant and organized in order to establish the diagnosis of LA or ACA.

Laboratory Diagnosis

The laboratory diagnosis of LA remains one of the most commonly encountered problems in the coagulation laboratory. Through the efforts of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies of the Scientific and Standardization Committee of the ISTH, some degree of interlaboratory consistency is emerging.⁽¹⁰⁾ Utilizing these criteria will greatly enhance interlaboratory reliability and consistency. When laboratories receive a request from a clinician to evaluate a patient for the presence of LA, it is imperative to test for both LA and ACA. These antibodies in many patients (~30%) are different (Figure 1).

APA Laboratory Evaluation



Triplett DA. Antiphospholipid-protein antibodies: laboratory detection and clinical relevance. *Thromb Res* 78: 1-31, 1995.

Figure 1. APA Laboratory Evaluation.

From: Triplett DA. Antiphospholipid-protein antibodies: laboratory detection and clinical relevance. *Thromb Res* 78: 1-31, 1995.

One of the most important aspects of testing for LA is proper preparation of platelet-free/platelet-poor plasma. This is necessary for fresh plasma, preparation of frozen samples or preparation of pooled normal plasma for mixing studies. Double centrifugation or filtration is the most common method for preparation of platelet-free plasma.⁽²⁸⁾

Two or more tests are necessary to screen for LA before the diagnosis is excluded. At least one of these tests should be based on a low PL concentration (dRVVT, KCT, dAPTT, dPT). The screening assay should represent different assay principles; for example, APTT and a dRVVT. If an abnormal screening procedure is identified, the remainder of the diagnosis should employ the same procedure for mixing studies and confirmation.

In order to make the diagnosis of LA, the following guidelines are recommended:

- Identification of an abnormal screening procedure utilizing a PL dependent assay system.
- Evidence of an inhibitory activity established by mixing studies (patient plasma/pooled normal plasma).
- Proof the inhibitory activity is PL dependent. This may be achieved by the addition or alternation of phospholipid concentrations, addition of hexagonal phase PL, or the use of platelets or platelet vesicles.
- Rule out other coagulopathies such as specific factor inhibitors.

Conclusion

APA may be encountered in many different clinical and laboratory situations. It is imperative for the laboratory to have a high degree of vigilance and an organized, thorough approach to establishing the diagnosis of LA/ACA. Close collaboration and cooperation with the clinician is critical in resolving many of the perplexing aspects of the laboratory diagnosis of APA. Adherence to the criteria for the diagnosis of lupus anticoagulants as outlined by the Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies is important in establishing a degree of consistency between laboratories. Utilization of these common guidelines will help in improving clinical data for interinstitutional studies.

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