Antiphospholipid-Protein Antibodies and Ischemic Stroke
Not Just Cardiolipin Any More

David Tanne, MD; Douglas A. Triplett, MD, FACP, FCAP; Steven R. Levine, MD

Within the past decade, cerebral infarction in as many as 40% of patients was not found to have a determined cause based on NINCDS Stroke Data Bank criteria. With improved understanding of the complex pathogenic processes leading to ischemic stroke and refined imaging and diagnostic tests, underlying potential causes are more often recognized. Yet, the etiology of ischemic stroke in a discouragingly large number of patients continues to elude clinicians.

Antiphospholipid antibodies (aPL) are a heterogeneous family of autoantibodies associated with a clinical syndrome characterized by thrombo-occlusive events. Anticardiolipin antibodies (aCL), detected by standard enzyme-linked immunosorbent assay (ELISA), and the lupus anticoagulant (LA), which prolongs phospholipid-dependent coagulation assays, are conventional assays for aPL and the ones currently best characterized and standardized. There is partial concordance between the 2 assays. The preponderance of evidence indicates, however, that LA assay is more specific for patients at risk for thromboembolic events. In contrast, the aCL assay is more sensitive but nonspecific and could be found also in various contexts ranging from health to certain medications, malignancies, and infectious diseases. aCL have been identified in approximately 10% of unselected patients with first malignancies, and infectious diseases. aCL have been identified in as many as 40% of patients who were not found to have a determined cause of their ischemic stroke.

The isotype mainly implicated in thrombosis is IgG, more specifically subtype IgG. Recent data suggest that the presence of high titers of aCL immunoreactivity, mainly IgG isotype but possibly also IgM, correlates with an increased risk of thrombosis. Generally, titers of IgG aCL implicated are >40 GPL, although this is a somewhat arbitrary cutoff point and is dependent on the test systems, which are not standardized.

Data accumulating over the last few years have radically changed our understanding of the antigenic specificities of the autoantibodies associated with the antiphospholipid syndrome (aPS) and the pathogenic mechanisms associated with these antibodies. The concept of a protein target for aPL evolved from a series of independent reports in 1990 that identified β2-glycoprotein I (β2-GPI; also named apolipoprotein H) as a necessary plasma cofactor to bind cardiolipin in vitro on ELISA plates. β2-GPI is a 50-kDa plasma protein that has several anticoagulant functions. Anti–β2-GPI antibodies, now well studied, can help differentiate between autoimmune aCL that require β2-GPI and “benign” alloimmune aCL that do not. In fact, β2-GPI is often inhibitory in the assay system rather than a positive cofactor in these cases. Anti–β2-GPI antibodies were shown to be more specific for thrombosis than conventional aCL and can occasionally be the only positive assay associated with the aPS. ELISA kits for antibodies against β2-GPI are currently available and FDA approved.

It soon became apparent that most autoantibodies detected in conventional aCL and LA assays recognize certain phospholipid-binding plasma proteins, not phospholipid alone. Other proteins implicated include prothrombin, protein C, protein S, thrombomodulin, annexin V, and kininogens. The majority of patients who manifest the LA contain a “cocktail” of antibodies, mostly antibodies to β2-GPI as well as antibodies to prothrombin and perhaps other plasma proteins. In most cases, LA activity found in a given patient is due to predominance of antibodies to prothrombin. Assays for antibodies against such specific plasma proteins may enable subclassifications based on the protein component of the protein-phospholipid complex, but currently they remain in the realm of development and research.

Antibodies against phospholipids other than cardiolipin have been less well studied and characterized than aCL. One reason is that there is extensive cross-reactivity of aCL with other negatively charged phospholipids. Whereas cardiolipin occurs primarily intracellularly, such as in the mitochondrial membrane, other phospholipids are important constituents of the cell membrane. Patients with clinical (and other laboratory) manifestations of the aPS may occasionally have persistently negative conventional assays for LA and aCL but positive for antibodies directed against other phospholipids. These include mainly anionic moieties such as phosphatidylserine and phosphatidylinositol and occasionally neutral phospholipids such as phosphatidylethanolamine. Preliminary data also suggest that antibodies directed against phosphatidylserine may react directly with central nervous system tissue and may be more specifically associated with ischemic stroke.
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An algorithm for testing for antiphospholipid antibodies. A suggested setting based on available data and authors’ clinical experience. Such as recurrent miscarriages, deep vein thrombosis, livedo reticularis, left-sided cardiac valve lesions or thickening, or systemic lupus erythematosus (SLE)/lupus-like disease. Such as thrombocytopenia, false-positive VDRL, elevated activated partial thromboplastin time, or antinuclear antibody. High sensitivity, low specificity. Likely autoimmune if IgG isotype, titer > 40 GPL, persistent after at least 8 weeks (thus requires retesting to assess for persistence). Highest specificity but low sensitivity. Under investigation; based mainly on data from patients with SLE or the aPS. Antibodies to β2-GPI are more specific for thrombosis when compared with aCL.

Thus, antiphospholipid-protein antibodies (aPL-P), rather than being a single or even a homogenous group of autoantibodies, constitute a heterogeneous family of autoantibodies with different isotypes, different specificities, different requirements of cofactor proteins, and different immunological characteristics. aPL-P may interfere with the kinetics of coagulation reactions or stimulate the prothrombotic activities of endothelial cells and monocytes and promote coagulation by complex molecular interactions. The specificity of different aPL-P to thrombosis in the venous and/or the arterial circulation remains a matter of investigation. aPL-P are likely associated with venous thromboembolism in approximately two thirds of cases, and in the other third of cases arterial events predominate. The interesting observation of the fidelity with which one sees recurrent events (ie, arterial event→arterial event, venous event→venous event) was first proposed by Rosove and Brewer.

Preliminary data suggest that immunological factors may contribute not only to thrombosis but also to atherosclerosis, mediated by aPL-P. Patients with aPS have increased levels of antibodies to oxidized LDL, associated with progression of atherosclerosis and risk of thrombo-occlusive events. Antibody responses to phospholipids, oxidized LDL, β2-GPI, prothrombin, and endothelial cells partially overlap and may reflect a broadening spectrum of autoantibody-associated atherothrombotic disease.

After a decade of research on the association between aPL and stroke, it is still unclear whether aPL are an intriguing but rare cause of stroke in young patients, play a pathogenic role in a large proportion of unselected ischemic stroke patients, or both. Patients with ischemic stroke are often elderly, rare cause of stroke in young patients, play a pathogenic role in a large proportion of unselected ischemic stroke patients, or both. Patients with ischemic stroke are often elderly, with multiple vascular risk factors, diffuse atherosclerosis, and cardiac impairment, and thus have potentially multiple underlying mechanisms for thromboembolism. Cardiovascular

In an interesting study reported in this issue of Stroke, a high prevalence of antiphosphatidylinositol antibodies was identified in a young population of cryptogenic stroke or transient ischemic attack patients. Antibodies directed to 7 different phospholipids were systematically assessed, and an especially high prevalence (44%) of antibodies directed to 1 or more of these phospholipids (dependent on β2-GPI) was found in this population. Furthermore, nearly one quarter of patients with negative immunoreactivity to aCL demonstrated positive immunoreactivity specific to noncardiolipin phospholipids. Among the aPL-P studied, those with specificity for phosphatidylinositol had the highest prevalence. This preliminary study suggests that by assessing only aCL and LA in young stroke patients, we may be underestimating the potential prevalence of aPL-P.

Caution should, however, be exercised in overinterpreting these provocative preliminary findings. The issue of phospholipid specificity for aPL is one that has not been appropriately addressed in the past. On the basis of the majority of available information, one would assume that β2-GPI represents the antigenic target in most cases in which we are dealing with negatively charged phospholipids applied to a microtiter plate. However, there is a distinct possibility that other plasma proteins may serve as “cofactors” for the phosphatidylinositol studies. Recent work on phosphatidylethanolamine would support this hypothesis. In the case of phosphatidylethanolamine, both high- and low-molecular-weight kininogens have been implicated as cofactors. Thus, antibodies to antiphosphatidylethanolamine would be missed in a test system that did not provide a source of kininogens. Do antibodies to phosphatidylinositol indeed have a specific protein cofactor? Does the participation of a specific phospholipid in a phospholipid-protein complex confer greater pathogenicity to the complex antigen?

It is imperative that studies on aPL-P include specific descriptions of the assays used because interpretation of any finding highly depends on the microtiter plates used (assays using oxidized or irradiated microtiter plates are made to be highly sensitive, presumably by reconfiguration of protein to expose a neotype), as well as other aspects of the procedure including buffers, blocking agents, and the presence of animal β2-GPI (eg, bovine). One of the major difficulties in the area of aPL-P is laboratory variability. Efforts to standardize assays are required to allow reliable comparison of results between studies and to enable any future useful implementation in clinical practice. Assaying in parallel for

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**Appropriate Clinical Setting**
- Stroke in the young or of undetermined origin
- Clinical or serological manifestations of the APS
- Recurrent strokes or thrombo-occlusive events

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**Consider:***
- Antibodies against β2-GPI or other specific proteins
- Antibodies against non-cardiolipin phospholipids

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**Antibody Responses to Phospholipids, Oxidized LDL,** and prothrombin is a predictor of myocardial infarction support an important role of aPL-P in the pathogenesis of thrombo-occlusive events. It is possible that the presence of certain aPL alters the threshold for thrombosis and thus creates a “permissive thrombotic environment.”

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**Additional Notes:***
- Recent reports demonstrating that the presence of antibodies against phospholipids, oxidized LDL, and prothrombin is a predictor of myocardial infarction support an important role of aPL-P in the pathogenesis of thrombo-occlusive events. It is possible that the presence of certain aPL alters the threshold for thrombosis and thus creates a “permissive thrombotic environment.”

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**References:**
antibodies against specific plasma proteins may help to clarify the specificity of such findings. Only carefully designed case-control and complementary prospective studies (or case-control studies nested in a prospective study) of sufficient statistical power, coupled with assessment of unselected ischemic stroke patients, will enable us to critically assess the role of these non–aCL \textit{aPLP} in ischemic stroke.\textsuperscript{55,56} Similarly, we look forward to results from the nested case-control analysis from the Honolulu Heart Study assessing \(\beta_2\text{-GPI–dependent aCL}\) and antibodies to \(\beta_2\text{-GPI}\) in stroke and myocardial infarction patients from this cohort (Steven J. Kittner, personal communication, April 1998).

Currently, aCL testing (using irradiated or highly sensitive microtiter plates) and evaluation for the LA following accepted criteria\textsuperscript{3} are the recommended screening tests. These tests may be useful only in appropriate clinical settings, as outlined in the Figure. Retesting for persistence of the antibody after at least 8 weeks is of great importance. Data based on patients with the aPS suggest that there remain approximately 10% to 15% of patients who, despite presenting the clinical picture of the aPS, have negative tests for aCL and LA. Thus, in patients with high clinical suspicion, further testing is indicated, such as antibodies to \(\beta_2\text{-GPI}^\text{GPI}\), possibly to prothrombin (however, this is somewhat controversial because there is no clear correlation between the presence of antibodies to prothrombin and thrombotic events), or to noncardiolipin phospholipids. Antibodies against \(\beta_2\text{-GPI}\) or other specific proteins may be used as more specific confirmatory tests in patients with positive aCL and potentially related thrombo-occlusive events.

It is important for the clinician to appreciate the test systems used by their local or reference laboratories and by their quality control systems. These promising immunoassays, however, must be standardized, their variability among different laboratories assessed, and their clinical utility in ischemic stroke established before they can be recommended for general use.

**Acknowledgment**

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