Anticardiolipin Test and the Antiphospholipid (Hughes) Syndrome: 20 Years and Counting!

It has been 20 years since *The Lancet* published the first report on the anticardiolipin (aCL) test that identified a group of patients subject to recurrent arterial and/or venous thromboses and recurrent pregnancy losses (RPL). It spawned a wave of interest in what came to be known as the antiphospholipid syndrome (APS).

In the early 1980s, Graham Hughes and his team published a number of studies that showed an association of aCL with stroke, deep vein thrombosis, RPL, livedo, seizures, chorea, renal and liver vein thrombosis, thrombocytopenia, and pulmonary hypertension. This was initially called the aCL syndrome, but as aCL were found to cross-react with other negatively charged phospholipids, it came to be known as the antiphospholipid (aPL) syndrome. Hughes pointed out that the syndrome could occur in patients who did not have systemic lupus erythematosus, and introduced the term primary APS for non-lupus patients at the British Society for Rheumatology meeting in 1987. The interest this subject has generated has amazed even the most optimistic of the original coauthors of the first *Lancet* report, which has now been cited more than 1100 times. There have been more than 6000 publications related to APS and aCL in the last 15–20 years and the syndrome now encompasses features as diverse as heart valve disease, avascular necrosis, dementia, atypical multiple sclerosis, graft rejection, skin ulceration, and accelerated atheroma.

The story of the aCL test has its origins in an *in vivo* clotting abnormality called the lupus anticoagulant (LAC). The term LAC is paradoxical, since it actually prolonged clotting *in vitro*, but bleeding was rarely attributed to the observed abnormalities, whereas thrombosis was said to occur in 20%–30% of positive patients. It was also known that the LAC was associated with a biologic false-positive test for syphilis (BFP-STS), suggesting that this clotting abnormality might be caused by antibodies binding to cardiolipin or related phospholipids. The LAC test was difficult to perform, not standardized, and probably not very sensitive, and it was likely that patients with the disorder were being missed. Nigel Harris and Azzudin Gharavi, both working at the Hammersmith Hospital in London, reasoned that a solid-phase immunoassay with cardiolipin as antigen would enable more sensitive detection of the LAC-related antibodies. This hypothesis proved correct, and their report in *The Lancet* showed an association of aCL positivity with thrombosis, pregnancy loss, and presence of the LAC. In addition, the finding of aCL-positive patients with clinical complications who were LAC-negative illustrated the greater sensitivity of the new test in identifying these patients.

In 1984, Hughes and colleagues sponsored the first international meeting on aPL and since then, 10 meetings have been held entirely devoted to this subject in cities throughout the world, attracting hundreds of physicians and scientists. Presentations on APS are now commonplace and draw large audiences at rheumatology, obstetrics/gynecology, hematology, neurology, and other meetings. APS is now said to be the most common cause of acquired thrombotic disorders and the commonest treatable cause of RPL.

The study of aPL has been full of surprises. In 1990, 2 groups found that most aPL did not only bind negatively charged phospholipids, but a serum protein termed ß2-glycoprotein I (ß2-GPI) was known to be a natural anticoagulant. The finding led to speculation that aPL might promote thrombosis by interfering with the anticoagulant properties of ß2-GPI. Since 1990, subsets of these antibodies have been shown to bind a host of other serum proteins, including prothrombin. Hence, aPL comprise a family of autoantibodies specific for a variety of serum proteins and phospholipids. Even more intriguing is the demonstration by Gharavi and colleagues that immunization of mice with ß2-GPI generated both anti-ß2-GPI and aCL. Subsequent studies showed that these mice were subject to enhanced thrombosis and fetal loss. As intriguing was the observation that bacterial and viral peptides homologous to amino acid sequences of ß2-GPI could induce aCL that also caused thrombosis and pregnancy loss. Together these findings are persuasive evidence for an infectious etiology for systemic autoimmune disorders.

The study of APS is not without controversy. The standardization and interpretation of aCL and LAC test results remain contentious even after 20 years of investigation. These have been the subjects of more standardization workshops than any other autoantibody test. The aCL test is very sensitive and patients with a variety of disorders can have false-positive results. Patients with APS tend to have higher
levels of the antibody (usually IgG) that persist for prolonged periods of time. To increase specificity for APS, it was reasoned that a measuring scale should be developed that would enable determination of aCL level by isotype (IgG, IgM, IgA). Utilizing a unique approach of mixing various quantities of positive and negative sera, a group of calibrators were developed from which aCL levels could be derived and results reported in specific units (for example, levels of IgG aCL are reported in GPL units). These calibrators were widely adopted and results reported in the specified units. However, because of limitations of the ELISA, there is wide variation between laboratories in measurement of absolute aCL levels. Better agreement occurs if results are reported semiquantitatively, i.e., high, medium, low, or negative. Although there is consensus about the steps to perform and confirm presence of the LAC, laboratories differ in the actual tests that make up their LAC panels, and as a consequence there are interlaboratory variations in results. Newer and more specific tests for APS such as the anti-ß2-GPI assay will probably also be subject to interlaboratory variation, representing the general limitations of the ELISA itself and how it is performed.

Unique animal models have been developed that provide insights into pathogenesis and treatment of the disorder. Several studies have shown that these antibodies enhance thrombus formation, activate endothelial cells, and cause pregnancy loss in mice. A variety of causal mechanisms have been proposed suggesting that these antibodies might induce expression of intracellular adhesion molecules [intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, E- and P-selectin] on endothelial cells, and these would have a procoagulant effect. Of note, ICAM-1 and E- and P-selectin knockout mice are not subject to aPL-induced thrombosis. Investigators have also reported that aPL enhance platelet activation and aggregation in vitro. Recent publications have shown that complement activation (more specifically C3 and C5) is required in aPL-mediated thrombosis and pregnancy loss. Other groups have suggested that pregnancy loss may be due to aPL inhibition of the anticoagulant effects of annexin.

The most meaningful consequence of this work is the difference it has made to patients. Several studies have now shown that oral anticoagulant therapy is effective in preventing recurrent venous and arterial thrombosis, although debate persists about the level of anticoagulation (international normalized ratio, INR) required to prevent recurrence. An aggressive form of the disorder manifests as multiple thromboses and organ failure, known as catastrophic APS. For affected women, pregnancy loss can be prevented in the majority with use of subcutaneous heparin and low dose aspirin. As experience has grown with this disorder, drugs such as hydroxychloroquine and statins have been shown in animal models to prevent aPL-mediated thrombosis and may be useful as prophylaxis in select groups of patients. Low molecular weight heparin is increasingly replacing use of unfractionated heparin to prevent RPL.

For those who have followed this story for the last 20 years, it has been an exciting journey. Hundreds of patients with unexplained venous and arterial thrombosis have been identified and morbidity and even death prevented by use of appropriate prophylaxis and monitoring. Similar numbers of women who may not otherwise have successful pregnancies have now been able to do so with appropriate treatment. We have learned through animal models that some autoantibodies probably play a direct role in pathogenesis of systemic autoimmune diseases, and there is new evidence for infectious agents playing a role in the causation of these disorders. Recently, interest has been directed toward the intracellular mechanisms involved in aPL-mediated platelet and endothelial cell activation and in the determination of the receptor(s) involved in these processes. Understanding these mechanisms better may result in the use of less toxic agents.

There are still many unanswered basic and clinical questions about APS, including disease mechanisms at the molecular level and whether or not minor manifestations, such as livedo reticularis, are predictive of more serious complications. The next 20 years should just be as exciting as the last.

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REFERENCES