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Anticardiolipin antibodies: Importance, controversies, discrepancies; the need for guidelines and calibrators

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Antiphospholipid syndrome (APS) is sometimes called the syndrome created by an assay. Serological reactivities due to antiphospholipid (aPL) antibodies were initially detected many years ago as biological false positive serological tests for syphilis (BFP-STS) (1) and lupus anticoagulant (LA) (2). Their presence in systemic lupus erythematosus (SLE) and other autoimmune conditions (3) and their association with thrombosis and recurrent pregnancy losses (4,5) have been suspected for the last fifty years. However, definitive recognition of aPL antibodies and the APS dates from 1983, with the introduction of a sensitive solid phase assay for the detection of the anticardiolipin (aCL) antibodies (6). The initial assay was a radioimmunoassay, and this was soon converted to an ELISA (7). The aCL ELISA was subsequently standardized in an international workshop in 1986 (8). The introduction of this sensitive and reproducible immunoassay revolutionized the field of antiphospholipid antibodies by facilitating the study of large numbers of patients. The associations of thrombosis, recurrent fetal loss, thrombocytopenia, and lupus anticoagulant with these antibodies were established, and the antiphospholipid syndrome or "Hughes Syndrome" (APS) was described (9-11). International consensus criteria have been published and tested, that clinically define APS in some detail in order to facilitate studies of the syndrome. Persistent presence of aPL antibodies of IgG or IgM isotypes (12) is a major laboratory feature of APS, and it is noteworthy that laboratory confirmation of APS may be based on elevated aCL alone, even in the absence of LA. Due to the life-threatening thromboembolic complications, the current treatment for definite APS is aggressive anticoagulation to achieve an INR of 3 or more (13). The duration of therapy of thromboembolic complications should be as long as aPL persist in plasma, and is often lifelong. Anticoagulation has a favorable effect on prognosis, but also involves a significant risk of serious bleeding. Therefore, a positive aCL test has a major potential impact on the patient's life and cannot be taken lightly.

During the first International Standardization Workshop for the Antiphospholipid Antibodies, the performance of the test in more than 30 participating laboratories was evaluated and some steps that could interfere with the validity of the test were identified. The pitfalls that were to be avoided included using Tween-20 in the washing, blocking or dilution buffers, or incubating the CL-coated ELISA plates at 37°C (8). The effect of incubation at 37°C was further investigated by Lockshin *et al.* (14). The use of 10% adult bovine serum or fetal calf serum, which are sources of $_2$glycoprotein I ($_2$GPI), in blocking and dilution buffers was also strongly recommended at that time (7, 8), when the essential role of the $_2$GPI in the aCL ELISA (15-17) was not yet known. Now, $_2$GPI alone or $_2$GPI /PL complex are recognized as the target antigen of aPL antibodies. Furthermore, aPL antibodies are sometimes referred to as anti-PL/ $_2$GPI or anti-$_2$GPI antibodies (15-17). At that workshop, aCL standards (calibrators), i.e. samples with predetermined aCL values for quantitation of the results, were also made available to laboratories interested in performing the aCL test in a valid and standardized way, and also to facilitate the comparison of assay results performed in different laboratories. In a second standardization workshop, the results of testing for aCL of samples distributed to many centers were compared and it was established that the inter-laboratory agreement was better when results were reported by ranges (high, medium, low) (18). Together these workshops intended to improve the agreement between aCL results obtained in different laboratories.

However, despite all these efforts to standardize the aCL ELISA there remains significant variation in the performance of the assay among laboratories, that no doubt contributes to the wide variation in prevalence of aCL reported among patients with SLE and SLE-like conditions (from 18% to 68%) (19). Coulam *et al.* (20), Peaceman *et al.* (21) and Favaloro *et al.* (22) documented large inter-laboratory vari-

ation in aCL testing. In a European multicenter study, with six participating centers the performance of nine commercial kits plus their in-house ELISA for aCL were evaluated. Agreement among the commercial kits was very poor. By contrast, the agreement among in-house assays was better, but far from satisfactory (23). The authors examined various assay conditions used in the commercial kits (use of detergent, incubation times, temperatures of incubation, etc) and concluded that some of the assays utilized the "non-recommended" procedures and that may have accounted for the differences observed (23). Recently, 30 European laboratories compared their aCL ELISA and the inter-laboratory agreement was not good (24). Again it was noted that when laboratories utilized "standard" procedures that conformed to proposed guidelines for aCL testing (what the authors called: "the consensus kit"), the agreement between centers was greatly improved, whereas agreement was poor among laboratories that used non-validated procedures.

Most of the participating laboratories used these assays for clinical diagnosis. What would be the outcome of using imprecise aCL assays for clinical diagnosis? Some patients might be anticoagulated unnecessarily while others who need anticoagulation might not receive it and therefore be at risk for thrombosis. The imprecision of the ELISA assay for aCL is therefore of some concern, and may to some degree reflect a problem common to most sensitive autoantibody assays. It must also be noted that very few, if any, autoantibody assays have been as extensively standardized by inter-laboratory testing as the aCL assay. However, much remains to be done to improve the clinical usefulness of aCL tests.

These controversies are not limited to clinical laboratories. In basic research as well, similar discrepancies have been noted. Hattori *et al*. reported the detection of these antibodies (the authors referred to them as anti-$_2$GPI) in the supernatant of peripheral blood mononuclear cells (PBMC) from APS patients after *in vitro* stimulation with $_2$GPI (25). In contrast, Dean *et al*. could not detect these antibodies in culture supernatants of PBMC from APS patients even after *in vitro* stimulation with $_2$GPI (26) [in the current issue of this journal]. Unlike the case of clinical laboratories that use the aCL test for APS diagnosis and treatment in patients, methodological discrepancies in basic research do not put the patient's life at risk. In most instances, the resulting scientific confusion stimulates further research and has generally been resolved by further detailed investigation.

In conclusion, introduction of the anticardiolipin test has helped many patients and saved many lives. However, many challenges remain to resolve the problems caused by the inconsistencies discussed above. A possible solution would be an ongoing forum to discuss the most common technical problems with the test, such as that started during the 1986 Standardization Workshop (8), and recently continued by the National Committee for Clinical Laboratory Standards (NCCLS) and the European Forum (24). The next APS Classification Workshop to be held in conjunction with the International Symposium on Antiphospholipid Antibodies, with the participation of the International Advisory Board, could also facilitate laboratories that wish to follow proposed guidelines for testing and utilize international calibrators and controls. Laboratories should also be encouraged to participate in a practical exercise that includes testing a panel of coded samples to determine their levels of aCL activity, similar to the one organized by the College of American Pathologists (CAP).

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