MANAGEMENT OF PRIMARY REFERENCE PROCEDURES FOR THE MEASUREMENT OF IGG ANTICARDIOLIPIN ANTIBODIES

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Abstract: Immunology laboratory departments in Romania have traditionally performed anticardiolipin (aCL) antibody assay to detect levels. Anticardiolipin antibodies occur during various autoimmune diseases, infectious diseases, neurological and kidney diseases, transplant loss, metabolic diseases, and drug abuse. They are also found in connection with reproductive failure. More than 30 years have passed since the first ELISA technique for aCL antibody detection was introduced to the clinical laboratory. The European Forum on APL antibodies has recently published guidelines for the management of aCL test in Thrombosis and Haemostasis. Continuous efforts are being made at international workshops for management standardization and to make it more specific.

Keywords: anticardiolipin antibodies, ELISA, specificity, management, technique.

Despite these efforts, in which improved anticardiolipin (aCL) ELISA kits were introduced, a considerable interlaboratory variation exists. The findings suggest that the aCL ELISA is neither very sensitive nor at all specific. Standardization is important because it facilitates clinical interpretation and comparison of results from various studies (Forastiero R. 2014, Pierangeli SS.2005, Abo SM,. 2007).

Test result for aCL antibodies alone is insufficient to establish the diagnosis of antiphospholipid syndrome (APS). It is essential to interpret results in the light of the patient's history and condition. The quantitative measurement of aCL antibodies is important in diagnosing APS (Bertolaccini ML. 2004, Audrain MA. 2004).

The predictive value of testing with the aCL ELISA for antiphospholipid syndrome (APS) can be improved by concurrent lupus anticoagulant (LA) testing. The European Forum on antiphospholipid (aPL) antibodies has recently published guidelines for the aCL test in Thrombosis and Haemostasis. Immunology departments in Romania have traditionally performed aCL antibody assay to detect levels of autoantibodies. Antiphospholipid syndrome is a relatively common disorder due to development of autoantibodies to cell membrane phospholipids (Miyakis S. 2006, Hoppensteadt DA. 2008).

Anticardiolipin antibodies occur during various autoimmune diseases, infectious diseases, neurological and kidney diseases, transplant loss, metabolic diseases, and drug abuse. They are also found in connection with reproductive failure. There is currently a wide range of automated ELISA systems being offered by a variety of companies. Laboratories tend to use automated systems because they are less labour intensive than manual assays; they can be loaded and then left alone until the assays have been completed. Although the initial costs of purchasing such a system are quite high, they are cheaper in the long run and are subject to less human error than manual assays (Ruffatti A, 2009).

More than 30 years have passed since the first ELISA technique for IgG aCL antibody assay was introduced to the clinical laboratory. However, standardization continues to be a major issue when comparing results. While more is known about pathogenic mechanisms and clinical significance of APL and more specific assays have been developed, significant performance differences still exist (Lakos G. 2011).

Antiphospholipid antibody assay standardization has been difficult to achieve due to differences in assay design (including reagent formulations and test procedures) and in the methods or materials used to calibrate the assays. In autoimmune serology, the quality of

samples (including sampling, patient preparation, optimal sampling time, transmission and processing) is the key of quality assurance. For any given serological test, sensitivity and specificity are determined by the cut-off (CO) value. A concentration above the cut-off point is often referred to as a "positive test result" and below the CO point as a "negative test result" (Tincani A. 2001).

We recommended a 4 fold increase of CO value to be clinically significant for an IgG aCL ELISA technique.

Most results are standardized by using GPL (IgG PhosphoLipid binding Units) for reporting IgG aCL levels: 1 GPL unit was defined as being equivalent to the binding of 1µg/mL of affinity-purified antibody. Most current aCL values are reported in arbitrary "units" rather than in µg/mL. We give a normal range of 20±15 GPL U/mL, with some abnormal results in APS patients over 100 GPL U/mL and very occasionally over 200 GPL U/mL (Gafou A. 2004).

Currently, serum aCL antibodies are determined by ELISA technique. The findings suggest that the aCL ELISA is neither very sensitive nor at all specific. There are some of laboratories that interpret results on a scale from negative through low titre, medium titre, to high titre positive. It is always interesting to take a look at how good (or bad) we are at what we do. There are many contradictions involved within the area of immunology testing with further complications arising where you compare results from different laboratories. Lock outlined the continuing problems with APL antibodies (Samarkos M. 2006).

The coefficients of variation between laboratories are typically between 25-30%. Almost all positive samples tested for IgG aCL antibodies show a full range of results from negative, weak and moderate positive to strong positive. Results vary with different assays and between laboratories. The immunoassay standardization is less well developed. The standardization is important because it facilitates clinical interpretation and comparison of results from various studies (Wisloff F. 2002).

This is especially important with analytes used for screening IgG aCL antibodies. ELISA technique has the advantage of being less subjective, more easily automated and less dependent on interpretation by experienced staff. However, there are serious concerns. Test results for aCL antibodies alone are insufficient to establish the diagnosis of a disease; they must always be interpreted in the clinical context. In other words, positive results may mean all sorts of things and can therefore be misleading. Firstly, they need to have good clinical reasons for requesting autoantibody tests (Tincani A, 2000).

It is also essential to interpret results in the light of the patient's history and condition. Both of these are problematic. Will the clinician understand the significance of the results and will they be able to interpret them in the clinical context? Autoimmune immunology can get pretty complicated and specialist advice is vital (Pierangeli SS. 2001).

These antibodies may be caused by infectious or non-autoimmune diseases unrelated to thrombosis. Most APS patients have multiple antibodies (polyclonal) that vary in specificity and affinity, as mentioned previously. Different scientific groups interested in standardization issues have also made recommendations on aCL antibody assay design, testing procedures, and the interpretation of assay results (Wong RCW. 2004).

In 2000, the National Committee for Clinical Laboratory Standards (NCCLS) proposed a guideline for the detection of aCL antibodies. The European Forum on aPL antibodies has made a consolidated effort to standardize aCL antibody testing. Although these suggestions are good laboratory practices that many laboratories currently perform. These include recommendations for additional testing with assays like (LA) or anti-beta glicoproteina 1 (anti-β2GP1) antibodies, the meaning of the various interpretative ranges and how they fit into the diagnostic criteria, with the recommendation that aCL antibody testing to be repeated in 6-8 weeks to determine if the levels are transient (Ruiz-Irastorza G. 2010).

Standardizing the assays would seems to be the best way to resolve the problems of the vast numbers of analysers and ELISA kits that are currently on the market.

Table 1. Anticardiolipin antibody ELISA test is positive in patients with a variety of other diseases

Autoimmune (SLE, autoimmune hemolytic anemia, autoimmune thrombocytopenia)
Viral (HIV, cytomegalovirus (CMV), HCV, Epstein-Barr (EBV), varicella-zoster, parvovirus B 19), Bacterial (spirochetes, tuberculosis, Lyme disease, Q fever, mycoplasma species, leprosy, Legionnaires's disease, Salmonella typhi)
Drug induced (chlorpromazine, procainamide)

Although these new and more specific tests have become available in the last 7-9 years, the aCL ELISA is the first choice. The newer tests might be used to confirm APS in patients in the following situations:

1. patients with the definite clinical criteria who are low positive (<40 GPL U/mL) IgG aCL antibodies;
2. patients with indefinite clinical APS criteria, or those in whom definite features may be attributed to factors other than APS;
3. patients negative for aCL antibodies and LA but with clinical features that are suggestive of APS (aPL antibody-negative syndrome) (Erkan D. 2011).

More efforts should go into standardization for quality assurance in aCL antibody testing. The aCL antibody assay is only one of the methods used to detect aPL, and the test should be administered with the LA and anti-β2GP1 antibody assays. The aCL antibody assay is reasonably sensitive but not at all specific; therefore, clinicians should treat the clinical state and not an incidentally found antibody. The diagnosis of APS is based on the demonstration of a moderate-to-high positive aCL antibody test (>40 GPL U/mL). Although there association between antibody titer and risk of thrombosis, this is not a ground for ignoring or reporting weakly-positive results. False-positive results that are difficult to interpret are particularly likely to occur when there are other causes of thrombosis such as atherosclerosis in the elderly**.** The predictive value of testing with the aCL ELISA for APS can be improved to extend by concurrent LA testing (Ruffatti A. 2008, Marjanovic S. 2005).

Conclusion

There is a lack of good guidelines both for the clinical aspects and the laboratory aspects of the antiphospholipid syndrome. The aCL antibody test is sensitive but not specific. One of the major drawbacks of the aCL ELISA test is false positive results.

Our data show that aCL ELISA standardization is necessary in order to obtain comparable results in different laboratories.

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