23 Anticardiolipin Testing

Silvia S. Pierangeli

Introduction

It is well known that patients affected with antiphospholipid syndrome (APS) are subject to episodes of thrombosis in arteries and/or veins, pregnancy loss (probably secondary to thrombosis of vessels in the placenta), and thrombocytopenia, associated with antiphospholipid (aPL) antibodies [1, 2]. aPL antibodies are autoantibodies directed against anionic phospholipids or protein–phospholipid complexes [3–5], measured in solid-phase immunoassays as anticardiolipin (aCL), or as an activity which prolongs phospholipid-dependent coagulation assays, the so-called lupus anticoagulants (LA) [6, 7]. Diagnosis of APS is based on finding a "moderate-to-high" positive aCL test and/or a LA test with any one of the characteristic clinical features presented above [2, 8].

The aCL test is important to aid the physician in diagnosis of APS [1, 8]. Although a sensitive test, aCL ELISA tests are positive in a variety of disorders, including connective tissue diseases, infectious disorders such as syphilis [9, 10], Q fever, and acquired immune deficiency syndrome (AIDS) [11–13], and some drug induced disorders [14]. It is generally believed that aCL are clinically significant only when present in APS; thus, there have been continuous attempts to modify the assay to make it *more specific* for APS. In addition, based on an early observation that patients with high positive IgG aCL tests were more likely to have APS [15], that ACL are heterogenous and measured using a wide variety of techniques, efforts have been devoted to quantifying the aCL ELISA test in a standardized manner [16–19]. This chapter will discuss the various techniques used in the diagnosis of APS, a series of aCL workshops that have been used to validate and improve measurement of aCL, as well as issues concerning problems and solutions concerning aCL testing. An overview of new and more specific tests for diagnosis of APS is also discussed.

Historical Background of the Anticardiolipin Test

In 1983, a group of investigators at the Hammersmith Hospital in London, England, noticed that some patients with systemic lupus erythematosus (SLE) had a rather uncommon coagulation abnormality called "lupus anticoagulant" [this was due to the abnormal prolongation of the partial thromboplastin time (PTT)] [20, 21]. The investigators also established that instead of abnormal bleeding, these patients were

subject to thrombosis [20]. Soon thereafter, the "lupus anticoagulant (LA) phenomenon" was known to be caused by an autoantibody believed to bind phospholipids, because they inhibited two phospholipid dependent coagulation reactions in the clotting-cascade – the prothrombin–thrombin conversion and the activation of factor X activation [22, 23]. In addition, about 25% to 50% of patients with the LA reaction also had a biological false positive test for syphilis (BFP-STS). Antibodies responsible for the BFP-STS were known to bind CL, a negatively charged phospholipid. The LA test had some drawbacks: it was a functional assay affected by a number of variables, including preparation and storage of samples, type of reagent use, etc. In addition, the test lacked sensitivity and could not be readily standardized. Thus, this group of investigators reasoned that use of a solid phase immunoassay with cardiolipin as antigen might be one way of detecting antibodies with LA activity [6]. They thought that such a test would have the advantages of greater sensitivity, more reproducibility, better quantitation, and the possibility of standardization. The group succeeded in establishing a solid phase radioimmunoassay with cardiolipin as antigen, and the antibodies were termed *anticardiolipin antibodies* (aCL) [6]. Hence, the first aCL test was established in 1983 [6]. The test proved more sensitive than the LA assay and enabled diagnosis of a much larger number of patients with APS. Also, the investigators soon noticed that aCL antibodies cross-reacted with negatively charged phospholipids, such as phosphatidylserine (PS) and phosphatidylglycerol (PG) [24]. Thus, the name aCL antibodies was changed to aPL [25]. And the disorder with which these antibodies were associated was called the antiphospholipid syndrome (APS) [25].

Widespread adoption of the solid phase aCL assay led to several potential problems. These antibodies were soon reported in several disorders, such as syphilis [9, 10, 26], AIDS [11, 12], connective tissue diseases, as well as in normal individuals who did not have the features of the disorder. "False" positive tests in the aforementioned conditions could be best explained by the sensitivity of the aCL test. However, methods of performing the test also varied and results were questionable in some instances. Fortunately, it was recognized that the majority of the patients with APS tended to have high aCL antibody levels, usually of the IgG isotype (however, some patients were only IgM positive) [27]. To ensure that the aCL test would retain its value in diagnosis APS, it would be necessary to identify antibodies by isotype and to quantify results using some reliable unit of measurement. There was also a need to establish which testing methods were valid as well as standard procedures for performing the solid phase immunoassay. To achieve these goals, several international standardization workshops were conducted following the first one in 1986 [16–19, 28].

Tests Used in Diagnosis of APS

The aCL Test

The aCL test was first established in 1983, using cardiolipin as an antigen, a mixture of gelatin/PBS to dilute patient serum, and radiollabeled anti-human IgG or IgM to detect bound aCL antibodies. The first aCL assay was a radioimmunoassay [6]. Since the test was first developed, several changes and improvements have been