

History of the Antiphospholipid Syndrome

Historia del Síndrome Antifosfolípido

Recognition of the Antiphospholipid Syndrome evolved over several decades, but unquestionably, the most exciting period was the decade between 1980 and 1990.

The origins of the syndrome dated back to the discoveries of the lupus anticoagulant by Conley and Hartmann in 1953, recognition of the association between the Biological False Positive Test for Syphilis (BFP-STS) and autoimmune diseases by Moore and Mohr in the 1950s. Subsequent investigators recognized over the next two decades that many patients with the lupus anticoagulant frequently had BFP-STS (much of this early history is summarized in my earliest review of the subject-Harris *et al*, *Clinics in Rheumatic Diseases*, 1985;11:591-609)

Initially, the lupus anticoagulant was thought to cause bleeding, but in 1964, Walter Bowie and colleagues reported a paradoxical association with thrombosis. The observation was extended by Johansson and colleagues in 1974, who recognized an association between the presence of lupus anticoagulant, a BFP-STS and thrombosis in patients with Systematic Lupus Erythematosus (SLE). In 1975, Nilsson and colleagues reported an association of the lupus anticoagulant and intrauterine fetal death.

Beginning in 1980, there appeared to be increased interest in this subject and during the three year period, 1980 to 1983, there were a number of reports of groups of patients with lupus anticoagulant, venous or arterial thrombosis and recurrent pregnancy loss. One center that took a particular interest in this association was that led by Dr. Graham Hughes, at the Hammersmith Hospital. In 1983, Meeling Boey and colleagues reported in the *BMJ* a series of SLE patients with lupus anticoagulant who were subject to thrombosis, recurrent abortion and/or thrombocytopenia.

A signal advance in understanding this disorder came with the introduction of the anti-cardiolipin solid phase immunoassay in 1983. Dr. Azuddin Gharavi, then a member of the group at the Hammersmith Hospital, postulated that antibodies responsible for the lupus anticoagulant test would bind cardiolipin, a negatively charged phospholipid, and that a solid phase radioimmunoassay would be more sensitive means of detecting these antibodies. This hypothesis was based on three pieces of data. The lupus anticoagulant was associated with the BFP-STS and antibodies responsible for the BFP-STS were thought to bind cardiolipin (the antigen bound by antibodies responsible for the VDRL test in syphilis). Secondly, the lupus anticoagulant test was "phospholipid dependent", such that the test was more prolonged when phospholipids in the test mixture was low, while on the



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apoya la década del hueso y la articulación

other hand, the test became negative when the concentration of phospholipids was increased (for many years, the lupus anticoagulant was referred to as an “anti-thromboplastin”). The third piece of evidence was based on the work of Thiagarajan and colleagues, who demonstrated that a monoclonal antibody with lupus anticoagulant activity bound negatively charged phospholipids in an Ouchterlony plate – subsequent work by the same group (led by Dr Sandor Shapiro) showed that polyclonal antibodies from patients with lupus anticoagulant interacted with negatively charged phospholipids in the same system (see my review in *Clin.Rheum Dis.*1985 for references).

I joined the Hughes group in early January 1983 as a Research Fellow and was immediately co-opted by Azzudin Gharavi to test the hypothesis that an anti-cardiolipin assay would be a more sensitive method of detecting these patients. Within about 2 months, we managed to develop a proto-type for an anti-cardiolipin radioimmunoassay. Fortunately, the Hammersmith group had access to sera of several patients previously reported by Boey et al; many of whom were lupus anticoagulant positive. As had been postulated, the overwhelming majority of patients (but not all) who were lupus anticoagulant positive had a positive anti-cardiolipin test. Of considerable importance, too, was that some lupus anticoagulant negative patients with clinical features of thrombosis, recurrent abortion and/or thrombocytopenia were also anti-cardiolipin positive. Anti-cardiolipin positivity was statistically associated with venous and/or arterial thrombosis, thrombocytopenia, and there was an equivocal association with pregnancy loss – these exciting findings were published in the November 26, 1983 issue of the *Lancet* (*this happy event was partly dependent on Graham Hughes visiting the then Editor of the Lancet to persuade him about the potential importance of this work*). An observation in that study that proved to be of later significance was that the higher the levels of anti-cardiolipin antibody positivity, the more likely the patient to have thrombosis or another of the associated clinical features – this came to mean that in later work to standardize the test, it would be important to devise a *reproducible means of measuring the level of anticardiolipin antibodies*). Soon after, the introduction of the anti-cardiolipin antibody assay, Graham Hughes proposed that the clinical features of this “disorder” did not only comprise thrombocytopenia, thrombosis, and pregnancy loss, but other features such as livedo reticularis and migraine headaches.

In the period between 1983 and 1987, there was increasing excitement globally about this unusual disorder. In 1984, our group organized The International Symposium on Antiphospholipid Antibodies at the Hammersmith Hospital and we invited everyone we knew in the field to participate (this was the first of the “Antiphospholipid Symposia”, held every two or three years to this day (the next meeting will be in Galveston, Texas in 2010 - these meetings have certainly played a major role in advancing knowledge and worldwide interest in this subject).

In the “early years” (1983 to 1987), we concentrated first on better understanding the specificity of anticardiolipin antibodies and in improving the test itself. We quickly found that anticardiolipin antibodies could be “absorbed” by adding negatively charged phospholipid liposomes to sera (). Then, we demonstrated that these antibodies bound ELISA plates coated with negatively charged phospholipids (such as phosphatidylserine and phosphatidic acid) as well as cardiolipin coated plates. These observations led us to conclude that “anti-cardiolipin” antibodies would be better referred to as “anti-phospholipid antibodies”, since the antibodies appeared to bind negatively charged phospholipids equally well. We expanded the term to include the lupus anticoagulant, which for reasons

cited previously was thought to have a similar specificity. By 1986, we stopped referring to these antibodies as “anticardiolipin” and used “antiphospholipid” instead.

As mentioned above, we worked also on improving the test method. Some investigators observed that use of Fetal Calf Serum or Adult bovine Serum as diluents in the anti-cardiolipin test greatly increased the positive signal - as if *enhancing the binding* of anti-cardiolipin antibodies. In addition, the assay proved more stable and reproducible with these diluents. It was not until 1990 that two groups determined that the serum protein, beta-2- glycoprotein1 was an important antigen recognized in the Fetal Calf or Adult Bovine serum diluent and this probably accounted for the observations noted.

Within the first two years of introduction of the assay and its adoption by laboratories worldwide, we became increasingly concerned that methods used to detect anti-cardiolipin antibodies varied considerably between laboratories and that some observations reported in the literature might be suspect. In 1986, this prompted us to conduct an International workshop to standardize the anti-cardiolipin antibody test (the results of the workshop were reported at the second Antiphospholipid Symposium held at St Thomas Hospital in London). The way that the workshop was organized was novel at time. The central thesis of the workshop was that laboratories doing the anti-cardiolipin test had to have a *valid assay that could reproducibly measure antibody levels*. Unlike most other efforts to standardize autoantibody tests, it would not be enough for participants to report a positive or negative result, but they were given a set of calibrators and their results (the readings they obtained for the calibrators) had to correlate with the “assigned values” of those calibrators. Calibrators were prepared by mixing a high positive serum with increasing quantities of normal human serum. The level of anti-cardiolipin antibody in the high positive serum had an assigned value in Units we defined, and the levels in the other mixtures (calibrators) calculated based on the proportion of the positive serum in the mixture. These levels were reported in GPL units (for IgG anti-cardiolipin), MPL units (for IgM anti-cardiolipin) and later IgA units (for IgA anti-cardiolipin) - these units have been widely adopted. We reasoned that a valid assay would be one that showed a “good statistical correlation” between the calculated value of calibrators and the optical density values obtained in the assay performed by the given laboratory. This approach was able to determine which laboratories had valid assays and which were the technical characteristics of the assay that enabled this- utilization of Fetal Calf or Adult Bovine serum as diluents of patient samples was found to be one very important feature.

By the mid-1980s, there was an increasing realization that this disorder was not a subset of SLE, but a separate entity (indeed, in a number of previous reports, particularly from hematology groups, many affected patients did not necessarily have SLE). An initial proposal was made to call the disorder the “anti-cardiolipin syndrome” but given evidence that the antibodies belonged to a larger family of “anti-phospholipid antibodies”, the term “Antiphospholipid Syndrome” was introduced. In 1987, in an editorial published in the British Journal of Rheumatology, I suggested criteria for classification of this “Syndrome” (The editorial was entitled “Syndrome of the Black Swan”). Patients were defined as having APS if they had at least one of the following clinical features – venous or arterial thrombosis, recurrent pregnancy loss and/or thrombocytopenia- and at least one laboratory test – lupus anticoagulant or a medium to high positive anticardiolipin antibody test. The laboratory test had to remain positive on, at least, two occasions eight weeks apart (this to distinguish the disorder from infectious illnesses in which “false positive” anti-cardiolipin or (less frequently) lupus anticoagulant tests could occur transiently).

Later, some groups proposed that APS be sub-classified into “primary” and “secondary” categories based on the absence or presence of SLE, respectively. This author has found little benefit in this sub-classification since in most cases there appears to be no difference in clinical or laboratory manifestations, prognosis nor required management (a similar conclusion was made two decades later by members of a workshop to review criteria for the disorder).

My initial suggestion of criteria was based on a “best guess” at the time. In subsequent years, there were two formal workshops of experts to establish and review criteria for APS (at Antiphospholipid Symposia in the late 1990s, in Sapporo and then at the Sydney conference in the early 2000s). In the main, several of the essential features of the first proposed criteria prevailed, but much more through definitions of what would constitute the clinical characteristics, laboratory features and addition of the anti-beta -2- glycoprotein 1 test now enables more reliable classification for clinical studies.

Recognition of a small subset of patients with an aggressive form of thrombosis at multiple sites occurred in the early 1990s. The name *acute disseminated vasculopathy* coagulopathy was first proposed by me in an editorial, but this gave way to the more user friendly nomenclature of “*Catastrophic Anti-phospholipid Syndrome*” proposed by Ronald Asherson. Collection of series of patients and a better understanding of the disorder is attributable to considerable subsequent the work of Ronald Asherson, Ricardo Cervera and Colleagues.

I moved to the USA (Louisville, Kentucky in 1987; then to Atlanta, Georgia in 1986). Aziz Gharavi had moved there just before I did.

During the 1990s and this decade after 2000, I have worked with Dr Silvia Pierangeli. Our interest in the standardization of the anticardiolipin test continued. We developed a variation of the test that uses a proprietary mixture of phospholipids (The Louisville APhL ELISA Test) that is distributed commercially as a kit. This particular assay, we believe enables a substantial decrease of “false positive” results one sees with the anticardiolipin assay. We have continued our interest in maximizing performance of this test and derivatives of the calibrators developed in the 1980s are still being distributed by the Antiphospholipid Standardization Laboratory now headed by Silvia Pierangeli.

With respect to Research work, in the last 20 years, Silvia Pierangeli and I have developed a mouse model of thrombosis in the APS and have conducted several studies (published extensively) on the role of antiphospholipid antibodies in thrombosis. This has evolved into work on mechanisms of thrombosis at the molecular level utilizing both the mouse model and in vitro studies.

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Indicaciones a los autores

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