

Clinical Studies Examining Thrombosis Related to Autoimmune Antiphospholipid Antibodies

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Abstract: Laboratory diagnosis of APS relies on the demonstration of positive antiphospholipid antibodies (aPL), including anticardiolipin antibody (aCL) and/or anti- β_2 glycoprotein I antibody (anti- β_2 GPI) by an in-house or commercially available enzyme-linked immunosorbent assay (ELISA) or lupus anticoagulant (LA) by a coagulation-based test. The relationship between aPL and thrombosis has been abundantly studied. However, the reported results, largely influenced by differences in study design, patient population and antibody assays, have been controversial and the precise serological 'fingerprint' of the patients most at risk of thrombosis remains elusive.

Keywords: Antiphospholipid antibodies, thrombosis, antiphospholipid syndrome.

INTRODUCTION

The antiphospholipid syndrome (APS), also known as Hughes syndrome, was first recognized in 1983 in patients with SLE [1]. The APS is an autoimmune disease where the most critical pathological process is thrombosis, which results in most of the clinical features suffered by these patients.

Arterial or venous thrombosis together with or without an adverse pregnancy history can be present. Any organ and any size of vessel can be affected; thus, the range of clinical features is extremely wide.

The risk of recurrent thrombosis is increased in patients with APS. The type of thrombosis is predictive; retrospective analysis of patients with APS and recurrent thrombosis showed that a venous thrombosis is followed by another venous thrombosis in more than 70% of cases, and an arterial thrombosis is followed by another arterial thrombosis in more than 90% of cases [2-4].

Laboratory diagnosis of APS relies on the demonstration of a positive anticardiolipin antibody (aCL) and/or anti- β_2 glycoprotein I antibodies (anti- β_2 GPI) by an in-house or commercially available enzyme-linked immunosorbent assay (ELISA) or on the presence of lupus anticoagulant (LA) by a coagulation-based test. It is important that these tests be performed in patients suspected of having APS where persistent positivity must be demonstrated and other causes and underlying factors considered. aPL are not uncommon. The prevalence of aPL among healthy donors has been reported to be around 10% [5, 6] whilst they have been found in about 25% of patients with unexplained venous thrombosis, 20% of patients with stroke [7], around 18% of patients with premature coronary artery thrombosis [8, 9] and in about 30% of patients with recurrent foetal loss [10].

CLINICAL STUDIES ON aCL, ANTI- β_2 GPI AND LA

The relationship between aPL and thrombosis has been abundantly studied. However, the reported results, largely influenced by differences in study design, patient population and antibody assays, have been controversial.

Early prospective studies in the general population have shown that aCL is a risk factor for first deep venous thrombosis [11] and recurrent venous thrombosis [12]. Finazzi *et al.* [13] designed a large prospective study in 360 unselected patients with LA with or without aCL and showed that IgG aCL above 40 GPL units and previous thrombosis were independent predictors of subsequent vascular thrombosis during a median follow-up of 3.9 years.

Although there is a high concordance between LA and aCL [14, 15], these antibodies are not identical. In general, LA is more specific than aCL for the APS though less sensitive. A meta-analysis of APS and the risk of venous thrombosis in SLE found that LA positive patients were 6 times more likely to have such an event whilst patients with aCL were 2 times more likely [16]. A subsequent meta-analysis of aPL and venous thrombosis in patients without underlying immunologic disease also concluded that LA was a more specific associate of thrombosis than aCL [17]. These findings were confirmed by a large prospective study where both the presence of LA and polyclonal aCL were associated with the risk of venous thrombosis, but LA was a better predictor of risk than was aCL [18].

Many studies have found that aPL are associated with an increased risk of cerebral ischemia [7, 19-23] but other studies have not reproduced these findings [11, 24, 25]. The Antiphospholipid Antibody and Stroke Study Group (APASS)/Warfarin Aspirin Recurrent Stroke Study (WARSS) collaboration evaluated the recurrent stroke risk attributed to aPL in a group of nearly 2,000 patients with ischemic stroke and suggested that aPL did not predict recurrent stroke in the general population [26]. It is important to mention that this study included patients with low titers of any isotype aCL

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(IgG, IgM and IgA) tested only at baseline, without any follow-up testing for persistence. Thus, this study does not address the question of recurrent stroke risk in patients with APS.

A number of studies - mainly in SLE patients - have confirmed the original observation of the association between aPL and seizures [27-29]. Seizures were found to be associated with moderate-to-high titres of aCL, suggesting a role for these antibodies in the aetiopathogenesis of epilepsy in SLE [28]. Our own experience confirmed a strong association between the presence of aPL and seizures in a large series of SLE patients [30]. Seizures in aPL-positive patients may be expression of ischemic events occurring as a result of hypercoagulability but there is also increasing evidence supporting the hypothesis of a direct interaction between aPL and neuronal tissue as a possible underlying mechanism [19, 31, 32].

In 2003, two systematic reviews addressed the association of the different aPL with thrombosis [33, 34] reporting that the LA was abidingly the most powerful predictor of thrombosis [33]. These data have now been supported by a large case-control study. Results from the RATIO study show that LA is a major risk factor for arterial thrombotic events in young women where the presence of other cardiovascular risk factors increases the risk even further [35].

A recent study by Galli *et al.* support the relation between anti- β_2 GPI and thrombosis in a (mainly) LA positive population, a fact that challenge the clinical significance of isolated anti- β_2 GPI as predictor of events [36] although it is well accepted that concomitant positivity for anti- β_2 GPI and LA or aCL seems to actually increase the risk of thrombosis [37].

Although the presence of aPL in SLE is not always associated with clinical symptoms of APS [38, 39], two cohort studies in patients with SLE have confirmed that patients with LA are at the highest risk of thrombosis. Patients with repeatedly positive medium to high level aCL are also more likely to suffer vascular events, however, those with intermittent positivity for aCL, even when positive more than two times (as required in the laboratory classification criteria for APS [40, 41]), are not at an increased thrombotic risk compared with aPL-negative lupus patients [42, 43]. Patients with triple positivity for anti- LA, aCL and anti- β_2 GPI tend to have higher and more stable aPL levels over the time [44].

A recent prospective cohort study found that aPL discriminates between those with and without new vascular events as early as the second year of follow-up and it is therefore a good predictor of imminent vascular events [45].

CLINICAL STUDIES ON OTHER aPL

The clinical utility of aPL antibody assays to phospholipids other than cardiolipin and to phospholipid-binding proteins other than β_2 GPI remains unclear [46]; the precise serological 'fingerprint' of the patients most at risk of thrombosis remains elusive [47].

Data on the clinical value of antibodies directed to prothrombin (another phospholipid binding protein) are contradictory. Antiprothrombin antibodies are heterogeneous and can be directed to prothrombin coated onto irradiated

plates (aPT) or to phosphatidylserine-prothrombin complex (aPS-PT) [48]. A recent systematic review showed no association between the presence of antiprothrombin antibodies and thrombosis, irrespective of isotype, site and type of event and the presence of SLE [34]. In our experience, antiprothrombin antibodies are frequently found in SLE patients and their presence is associated with APS [48]. Most significantly, a few patients with aPL-related clinical features, who are negative for aCL, LA and anti- β_2 GPI had antiprothrombin antibodies either by the aPT or the aPS-PT assays, suggesting that testing for these antibodies could be of clinical benefit in patients who are negative for the routine testing [48, 49].

Antibodies to phosphatidylethanolamine (aPE) have also been reported as markers of the APS, particularly in those patients who are negative to routinely tested aPL. Sanmarco *et al.* [50] reported aPE in 15% of thrombotic patients compared to 3% of controls. Using a multivariate analysis in a case-control subgroup study, IgG aPE was found to be associated with venous thrombosis. Interestingly, 63% of the aPE positive patients were negative for aCL, anti- β_2 GPI and LA.

A number of other autoantibodies has been reported in patients with APS, including antibodies to annexin V [51, 52], high and low molecular weight kininogens or, less frequently, prekallikrein and Factor XI [53, 54]; to vascular heparan sulfate proteoglycan [55] heparin [56], factor XII [57-59] and thrombin [60]. Some data suggest that autoantibodies could be directed against components of protein C pathway [61], which includes protein C [62], protein S [63, 64] and thrombomodulin [65].

IgA ISOTYPE

There is still controversy as to whether patients with features of the APS, negative to IgG or IgM aPL, have IgA aPL and, if so, their clinical significance [66]. It has been reported that IgA is the dominant isotype in Afro-Caribbeans [67], and also in Afro-Americans [68]. However, IgA aPL are usually present at low or moderate titres, and they are sometimes transient. Moreover, they do not seem to be associated with clinical manifestations in these ethnic groups.

CONCLUSION

The debate regarding the clinical significance of aPL is still open and many variabilities between studies have been reported. The heterogeneity of aPL assays, the definition of positivity and the absence of appropriate control groups have lead to the report of controversial results.

Routine measurement of aCL, anti- β_2 GPI and LA is required to establish the thrombotic risk of patients with previous thrombosis. However, there is still a need for definitive evidence-based studies on the risk of thrombosis in aPL positive patients without previous thrombotic history along with a clear need for standardization of the routinely used and the development of more specific laboratory techniques to identify patients at particular risk.

The association of other aPL with APS and their clinical significance is far from being known amid that these tests are far from standardised. Their application should be restricted only to research rather than to routine diagnostic use.

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