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Antiphospholipid Antibodies in Lupus: An Update

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Abstract

Antiphospholipid antibodies (APLA) represent a heterogeneous family of autoantibodies (Ab), frequently detected in autoimmune diseases such as systemic lupus erythematosus (SLE), and are thought to play an active role in the development of thrombosis and/or fetal loss. These Ab react with negatively charged phospholipids, phospholipid/cofactor complexes, and plasma proteins. Detected in up-to 50% of SLE patients, beta 2-glycoprotein I (β2-GPI) dependent anti-cardiolipin (CL) Ab is recognized as risk-factors for thrombosis. However, aCL/β2-GPI Ab detection is challenging since comparisons between assay results are difficult. Developments are proceeding to reduce these differences, with the final objective being to better predict the risk of thrombosis (either first or recurrent) and fetal loss.

Historical Points

The history of antiphospholipid (aPL) autoantibodies (Ab) dates back to 1906 when Wasserman applied the complement fixation reaction to the diagnosis of syphilis as described by Bordet and Gengou and used as an antigenic source, fetal liver with congenital syphilis [1]. In the following year, Landsteiner demonstrated that an alcoholic extract from beef heart can replace the infected organ leading to the development of the Veneral Disease Research Laboratory (VDRL) test. In 1941, Pangborn showed that the main constituent of this extract was a mitochondrial phospholipid that was referred to as "cardiolipin" because of its cardiac origin. The massive use of the VDRL assay led to the observation that patients with autoimmune diseases and in particular those with systemic lupus erythematosus (SLE) had "false-positive syphilitic serology" [2]. At the same time, Conley and Hartmann described the presence of a "circulating anticoagulant" referred to later as "lupus anticoagulant" (LA) following the extension of the *in vitro* coagulation clot assay in SLE patients [3]. In 1963, Bowie et al. described for the first time the paradoxical association of the LA with thromboses but not with hemorrhages as might have been suggested by the extension of coagulation tests [4]. The association of repeated miscarriages and thrombotic events in the presence of LA is described in 1980 by Soulier and Boffa [5].

In 1983, Harris et al. developed a radioimmunoassay (RIA) and two years later an enzyme-linked immunosorbent assay (ELISA) for the detection of aCL Ab [6,7]. This led in 1987, to the first definition of the "antiphospholipid syndrome" (APS) as a clinical-biological entity associating at least one clinical manifestation with a biological anomaly [8]. In 1988, Asherson differentiated primary APS from SLE as he observed similar clinical and laboratory manifestations in non-lupus patients. The 1990s were marked by the discovery that aCL Ab were not directed against the cardiolipin per se, but against cofactors: beta 2-glycoprotein 1 (β2GP1) first, which was discovered separately by three teams; and next, prothrombin which are all part of the antiphospholipid autoantibody (APLA) family [9]. In 1995, Matsuura first described that aβ2-GPI Ab can recognize β2-GPI even in the absence of phospholipids.

Autoantigen and Epitopes

Phospholipids

Phospholipids incriminated in APS are present at the plasma membranes, are organized in bilayers and are classified according to their charge at physiological potential of hydrogen (pH). This charge can be neutral such as phosphatidylethanolamine (PE), sphingomyelin and phosphatidylcholine, or negative such as phosphatidylglycerol (cardiolipin), phosphatidylinositol, and phosphatidylserine.

Beta-2glycoprotein I

The main cofactor of aCL is β2-GPI or apolipoprotein [10]. β2-GPI binds to negatively charged molecules such as phospholipids, lipopolysaccharides, heparin, some lipoproteins, plastic after irradiation, and to cell membranes of activated platelets and endothelial cells. β2-GPI is a 50kDa single-chain polypeptide synthesized by the liver, and present in the plasma of normal subjects at an average concentration of 200 µg/mL. This circulating protein consists of a single polypeptide chain of 326 amino acids organized into 5 repetitive structures or "sushi domains" as shown in Figure 1A. The main binding sites for anionic phospholipids are located on the 5th domain (282KNKEKK287), recognized by infectious Ab and while aβ2-GP1 Ab associated with thrombosis are located on the first domain [11]. Dimerization of β2-GPI on the cell surface, following aβ2-GP1 Ab binding, leads to a prothrombotic state either by the inhibition of anticoagulant proteins (activated protein C, annexin V), or by the expression of adhesion molecules on the surface of endothelial cells [12].

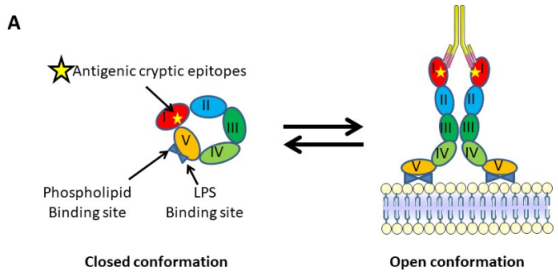

Figure 1A: The beta 2-glycoprotein I (β2-GPI) protein possesses two forms: a closed form and, after binding with anionic phospholipids or lipopolysaccharide (LPS), an activated and linear form. The conformational change of the molecule from a circular to a linear form is critical for exposing the cryptic epitopes present in domain I and then permitting pathogenic anti-(a) β2-GPI Ab recognition, which in turn triggers β2-GPI dimerization and activation of pathogenic cellular pathways.

β2-GPI is purified from normal plasma by chromatography (cardiolipin affinity or ion-exchange) or by sequential perchloric acid preparation. The purification step as well as the binding on the support is critical as it may influence β2-GPI structure and cryptic epitope accessibility as shown in Figure 1B. For the same reason it's not recommended to use recombinant proteins.

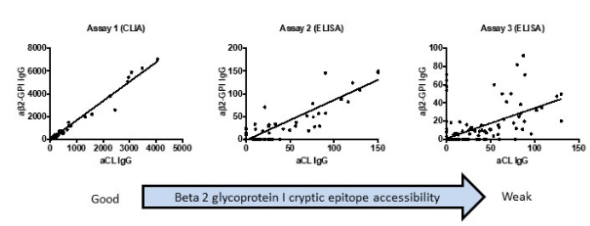

Figure 1B: In the immunological assays used for aβ2-GPI Ab detection, β2-GPI binding and cryptic epitope exposure is not always optimal as highlighted in these three assays by comparing aCL/aβ2-GPI results obtained using either magnetic beads (assay 1, chemiluminescent immunoassay [CLIA]), or irradiated polystyrene surfaces (Assays 2 [QUANTA Lite, Inova] and 3 ["in house" assay], enzyme linked immunosorbent assay [ELISA]), see [38] for more details. Results are expressed in GPL/mL for aCL IgG and in Units/mL for aβ2-GPI IgG.

Other Targets

The APLA family is not restricted to aCL and aβ2-GPI Abs. Other targets of APLA are related to prothrombin, protein C/S, oxidized lipoproteins, annexin V, thrombomodulin, high/low molecular weight kininogens, factor XII, complement component H and C4b, factor VII(a), and endothelial protein C receptor. The clinical significance of Abs other than LA, aCL/aβ2-GPI Abs, and anti-phosphatidyl serine/prothrombin (aPS/PT) have never been consistently and repeatedly associated with thrombosis and/or fetal loss.

Pathogenic Role

From the in vitro studies, two additional pathogenic mechanisms ("two hit-models") are schematically retained: first, the aPL-dependent mechanisms that involve the activation of several cellular actors (endothelial cells, platelets, lymphocytes, monocytes) and on the other hand independent aPL mechanisms that involve the coagulation and fibrinolysis pathways [13].

After being activated by APLA, endothelial cells adopt a pro-thrombotic, pro-inflammatory, pro-adhesive phenotype and are capable of inducing chemokines involved in monocytic recruitment [14]. Activation of the endothelial cells is carried out via different cell surface receptors (annexin A2, Toll like receptors...) [15]. Monocytes and activated endothelial cells produce pro-thrombotic molecules and, in particular, tissue factor which is the main activator of the intrinsic pathway of coagulation [16]. The platelets are themselves activated via the interaction of β2-GPI with the apolipoprotein E receptor 2 (apoER2) and Gp1b receptors [17-21], which causes (in the presence of APLA) an increase in the expression of the GPIIb/IIIa complex, and increased production of thromboxane A2 and B2 potent pro-aggregating agents and vasoconstrictors [22].

Several studies have shown a consumption of complement proteins during and outside thrombotic processes during APS [23]. Mice deficient in C3, C5 or C5a receptors are protected from fetal loss induced by the injection of APLA [24]. Similarly, inhibition of the complement cascade by using an anti-C5 monoclonal Ab was effective for thrombosis prevention in an APS mouse model [25]. In addition, complement activation leads to the release of pro-inflammatory C3 (C3a) and C5 (C5a) anaphylatoxins, which have also been implicated in placental lesions observed during fetal loss.

Inhibition of natural anticoagulant activity was the first identified pro-thrombotic mechanism of APLA [26,27]. In fact, APLA alter the activation of protein C, as well as the ability of activated protein C to inactivate coagulation factors V and VIII [28]. They also inhibit other coagulation regulatory factors such as heparin cofactor II [29], antithrombin III, and tissue pathway factor inhibitor (TFPI) [30]. These activities are mediated by APLA directed against β2-GPI and/or prothrombin [31]. Finally, APLA reduce the physiological anticoagulant activity of annexin V, which increases the overall pro-coagulation state. Annexin V is a protein (concave disc shape) with a binding domain for phospholipids, synthesized by endothelial cells and trophoblast, which prevents interactions with coagulation/fibrinolysis proteins on the surface of the cells. In the presence of aCL/aβ2GP1 Abs, the annexin V network is broken. This is because the annexin V network has lower affinity for phospholipids compared to the antigenic complex β2-GPI/Ab on the surface of endothelial cells. This disruption of the annexin V network exposes the tissue factor and procoagulant phosphatidylserine [32] and thus the activation of the coagulation cascade whose ultimate consequence will be the occurrence of thrombotic events. In addition, the loss of annexin V, induced by APLA, on placental trophoblasts is a potential mechanism of fetal loss [33].

By blocking the cofactor effect of β2GPI on the main activator of fibrinolysis, the tissue-type plasminogen activator (t-PA), APLA and more particularly those directed against β2-GPI can thus interfere with the transformation from plasminogen to plasmin which ultimately promotes the maintenance of a prothrombotic state [34].

Detection: Sensitivity and Specificity

In the first-generation ELISA tests developed for APLA detection, most of the β2-GPI (not known at that time) was provided by the animal serum used for coating which introduced the risk of detecting animal xeno-aβ2-GPI Ab and in turn could cause false positive reactions. In the second generation assays, since 2000, the animal β2-GPI was replaced by human β2-GPI in order to detect human β2-GPI-dependent aCL Ab, which are more predictive of thrombotic risk.

In 1995, Matsuura was the first to describe that aβ2-GPI Ab are able to recognize β2-GPI even in the absence of phospholipids. In the same year, Roubey observed the necessity to use irradiated polystyrene ELISA plates in order to fix β2-GPI on a negatively charged surface. This increases the density of β2-GPI fixed on the plate, unmasks cryptic β2-GPI domain I epitopes and thus allows the detection of the aβ2-GPI Ab in the absence of phospholipids.

The current tests [35] recognize three kinds of aCL Ab but without distinguishing them. First, "true" aCL Abs that recognizes cardiolipin independently of β2-GP1 and are found in infections. They are mainly of IgM isotype and are generally present transiently (8-10 weeks) while for some chronic infections (e.g. HIV, leprosy) they can persist over time [36,37]. Second, APLA that recognize domain I of β2-GPI, they are the most pathogenic and are found in APS. Their presence is stable over time, present at high levels and mostly of the IgG isotype, which may be associated with the presence of IgM. It should be noted that in its inactive and soluble form, the domain I of β2-GPI is not accessible to aβ2-GPI Ab and that β2-GPI must be associated with phospholipids for cryptic epitope presentation. Third, APLA recognizing the other domains of β2-GPI and in particular domain 5 whose pathological significance is not established.

The ELISA tests for APLA detection show numerous discrepancies between them because of the lack of efficient standardization. These differences can be explained by the methods used in the preparation of the cardiolipin antigenic mixtures / protein cofactors, by the use or not of irradiated plates (to improve the fixation of the antigens and domain I presentation), by the nature of the buffers used for the saturation of the plates ("Coating") and washes, and by the nature of the source of β2-GPI (purified human β2GP1 are preferred rather than animal serum). To circumvent these limitations new assays are in development in order to improve cryptic β2-GPI domain I epitope recognition by using either a new solid surface for β2-GPI binding or using specific β2-GPI domains by ELISA [37,38]. Last but not least, these tests do not detect aPS/PT Ab that is detected by a specific ELISA [39-41].

Clinical Associations and Limitations

In the general population, the prevalence of APLA ranges from 1 to 5% and increases with age since prevalence can reach 50% after 70 years [42]. Such positivity is in most cases at low-titer, of IgM isotype, and without dependence on β2-GPI. When the LA detection assay is positive, SLE and APS should be explored for this age group [43]. Isotype for APLA is important as IgG aCL/aβ2-GPI are considered to have a pathogenic role in thrombosis, such an association is also suspected for IgA but some doubts remain with regards to IgM. A study conducted under the APS ACTION group (Anti-Phospholipid Syndrome Alliance for Clinical Trials and International Networking) estimates that APLA are 13% of patients with stroke, 9.5% in those with deep vein thrombosis (DVT) and 6% in patients with obstetric morbidity [44].

Regarding APS, there are two forms of APS, one primary (APS-I) in 53% of cases and one secondary (APS-II) when it is associated with an autoimmune pathology (47%), more often SLE in 36% of those cases [45]. In SLE, APLA prevalence is high and ranges from 20 to 44% for aCL/aβ2-GPI Ab and 15 to 34% for LA [46]. In APLA positive SLE patients, the thrombosis risk is 4%/year versus 0.1%/year in the general population. The risk of thrombosis increases with the duration of SLE with thrombotic events reported in 50-70% of SLE patients, according to a 20 years follow-up study [46]. Some reports have described important heterogeneity in the prevalence of aCL Ab in SLE with values ranging from 2% in Afro-Caribbean to 51% in India, such variations are mainly explained by differences in the assay used and this should be reevaluated in the future. In children, the APS cases are exceptional but can concern 9% of the pediatric series of LES [47]. Magnetic resonance imaging (MRI) brain abnormalities are more common in SLE patients with APLA [48-50]. The prevalence of thrombocytopenia is higher in SLE with secondary APS than in primary APS. Finally, for about 1% of patients with APS-I/II, a very severe form called catastrophic antiphospholipid syndrome or CAPS is associated with very high mortality rates (35-50%). The average age of CAPS onset is 37 years with a female sex ratio of 3 according to the CAPS register [51].

More than just the simple analysis of the aCL/aβ2-GPI IgM/G positivity, the APLA profile can be used to predict thrombosis and pregnancy loss in primary and secondary APS as shown in Figure 2. Indeed, aCL/aβ2-GPI IgG double positivity and at high levels better predicts thrombosis than aCL/aβ2-GPI IgM or isolated/low level APLA. Such risk is further increased when associated with LA (triple positivity), aPS/PT, and APLA-independent risk factors such as hyperlipidemia, arterial hypertension, infections, oral contraception, surgery, immobilization, and inherited thrombophilia.

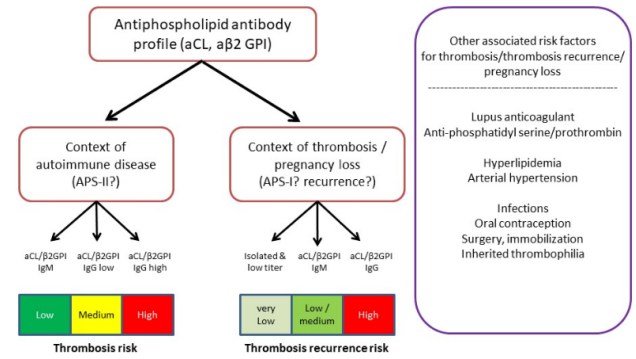

Figure 2: The risk of thrombosis (either first in patients with autoimmune diseases or recurrent) is multifactorial including the antiphospholipid antibody profile and independent risk factors.

Other Associations

The β2-GPI binds not only phospholipids but also other ligands such as oxidized LDL (oxLDL), and Abs against oxLDL/β2-GPI complexes strongly correlates with thrombosis and atherosclerosis in patients with SLE and APS. In addition, lipid peroxidation (oxidative stress) is common in SLE and APS, and circulating β2-GPI/oxLDL complexes are detected in these patients. Anti-oxLDL/β2-GPI Abs is

elevated in secondary APS patients (75%) compared to less than 40% in SLE patients without a history of thrombosis/fetal loss.

There seems to be a genetic predisposition, since some familial forms have been described without identifying the predominant candidate genes [52]. The most consistent associations with aCL/β2-GPI Ab and LA in SLE are those with human leukocyte antigen (HLA)-DR4, HLA-DPB1 and HLA-DRw53.

Conclusion

APLA can be detected in association with other autoimmune diseases, most frequently SLE. The prevalence of APLA in SLE ranges from 20-50%, among them half will develop thrombosis within 5 years, and thrombosis represents one of the most frequent causes of death (26.5%) during SLE follow-up [53]. As a consequence, this emphasizes the importance of exploring new detection methods for thrombosis prediction in SLE. With regards to aCL/β2-GPI Ab capacity to predict thrombosis (odds ratio: 3-4), important variations are described and these variations are partly explained by differences in the assays used. Then new technical developments are necessary to reduce these differences which could be attained in part by shifting from ELISA to other assay types such as chemi-luminescence assays as recently demonstrated by our group [38] and highlighted in Table 1.

	Chemiluminescence	**ELISA**
Technology	Direct binding on magnetic beads	Direct binding on irradiated plastic
Conjugated	Anti-IgG/M with ester acriddinium	Anti-IgG/M with enzyme
Calculation	Linear scale	Log scale
Results	Relative lights units	Optical density
Limit of detection	<1 unit/mL	<5 unit/mL
aCL IgG	Sensitivity 61% Specificity 100%	Sensitivity 56-62 Specificity 99%
aCL IgM	Sensitivity 23.7% Specificity 99.6%	Sensitivity 19-32% Specificity 99%
aβ2GPI IgG	Sensitivity 63.2% Specificity 100%	Sensitivity 36-47% Specificity 99%
aβ2GPI IgM	Sensitivity 25% Specificity 99.6%	Sensitivity 26-40% Specificity 99%
Thrombotic risk prediction (Odds ratio, OR)	aCL+aβ2GPI IgG: OR 3.39 aCL IgG high : OR 7.84 aβ2GPI IgG high: OR 7.07 aCL+aβ2GPI IgM: OR 0.25	aCL+aβ2GPI IgG: OR 3.4-4.4 aCL IgG high: OR 4.4-5.6 aβ2GPI IgG high: 4.4-5.6 aCL+aβ2GPI IgM: OR 0.3-0.6

Table 1: Comparison between chemiluminescence and Enzyme linked immunosorbent assay (ELISA) for anti-cardiolipin (CL) and anti-β2 glycoprotein I (GPI) determination [38].

Conflict of Interests

The authors have declared no conflicts of interest.

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