Recent advances in understanding antiphospholipid syndrome
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Abstract

Antiphospholipid syndrome (APS), also known as Hughes Syndrome, is a systemic autoimmune disease characterized by thrombosis and/or pregnancy morbidity in the presence of persistently positive antiphospholipid antibodies. A patient with APS must meet at least one of two clinical criteria (vascular thrombosis or complications of pregnancy) and at least one of two laboratory criteria including the persistent presence of lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and/or anti-b2 glycoprotein I (anti-b2GPI) antibodies of IgG or IgM isotype at medium to high titres in patient’s plasma. However, several other autoantibodies targeting other coagulation cascade proteins (i.e. prothrombin) or their complex with phospholipids (i.e. phosphatidylserine/prothrombin complex), or to some domains of B2GPI, have been proposed to be also relevant to APS. In fact, the value of testing for new aPL specificities in the identification of APS in thrombosis and/or pregnancy morbidity patients is currently being investigated.
Introduction
Antiphospholipid syndrome (APS), also known as Hughes Syndrome, is a systemic autoimmune disease characterized by thrombosis and/or pregnancy morbidity in the presence of persistently positive antiphospholipid antibodies. When APS was first described, it was in the presence of systemic lupus erythematosus (SLE); however, APS is now accepted to be a primary autoimmune syndrome with other accompanying characteristics, such as thrombocytopenia, seizure disorder, cognitive dysfunction, livedo reticularis, and renal vasculopathy, being frequent in the absence of the main clinical manifestations of thrombosis and pregnancy complications.

In 1999, definitive classification criteria for APS were published in an international consensus statement and subsequently revised in 2006. A patient with APS must meet at least one of two clinical criteria (vascular thrombosis or complications of pregnancy) and at least one of two laboratory criteria including the persistent presence of lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and/or anti-β2 glycoprotein I (anti-β2GPI) antibodies of IgG or IgM isotype at medium to high titres in patient’s plasma.

While it is widely accepted that the LA is the most important predictor for thrombosis, several other autoantibodies targeting other coagulation cascade proteins (i.e. prothrombin) or their complex with phospholipid (i.e. phosphatidylserine/prothrombin complex), or to some domains of β2GPI, have been proposed to be relevant to APS. In fact, the value of testing for new aPL specificities in the identification of APS in thrombosis and/or pregnancy morbidity patients is currently being evaluated, which will be especially useful for those with recurrent negative results in present tests.

New aPL specificities
Antibodies directed to the domain I of the β2GPI
β2GPI was identified as a primary target of autoantibodies in patients with APS. β2GPI is a single-chain protein containing five repeating sequences or domains. Domain V is essential for binding to anionic phospholipid membranes, whereas domain I sticks out into the extracellular space where interactions with other proteins/antibodies can take place. The development of recombinant domain specific β2GPI molecules by Iverson et al. in 1998 steered us towards a better understanding of the specific role of the autoantibodies to each of the five β2GPI domains. Several studies have detected antibodies recognizing various domains of β2GPI. However, anti-domain I (anti-DI) antibodies were frequently found to be highly associated with clinical symptoms and therefore focused upon.

In their 2005 study, de Laat et al. reported that patients testing positive for anti-DI had a higher thrombosis risk. Antibodies recognizing epitope G40-R43 on the domain I of β2GPI caused LA and strongly correlated with thrombosis. A larger, multicentre study in 2009 looked at a large cohort of anti-β2GPI positive patients, showing that those patients who were IgG anti-DI positive had a 3.5 fold increase in the risk of developing vascular thrombosis and a 2.4 fold increase in the risk of developing pregnancy morbidity when compared to those who tested negative for IgG anti-DI. Using inhibition assays, Banzato et al. demonstrated that high-risk patients, those bearing triple aPL positivity for aCL, LA and anti-β2GPI, are those with substantially greater titre of circulating anti-DI antibodies. Those with double and single positivity showed low titre or absence of anti-DI antibodies. Conversely, when tested on 326 patients with SLE, of whom 164 had a history of thrombosis, Akhter et al. failed to find an association between anti-DI and these events.

The domain profile of anti-β2GPI antibodies has also been explored in a large cohort of patients. While neither anti-DI nor anti-DIV/V antibodies were found to be associated with thrombotic events or obstetric morbidity, Andreoli et al. suggested that utilizing the ratio of anti-DI/anti-DIV/V could be useful as a biomarker for APS, identifying “pathogenic” from “non-pathogenic” anti-β2GPI. A recent study in aCL and/or aβ2GPI positive patients suggests that the added finding of anti-DI positivity makes it three to five times more likely to confirm APS. Positivity for IgG or IgA (but not IgM) anti-DI increased the strength of association between aCL/aβ2GPI and thrombotic manifestations in APS.

Anti-DI antibodies have also been reported in pediatric populations. Wahezi et al. reported a prevalence of IgG anti-DI of 25.1% in children with SLE. However, only seven children had thrombosis, failing to ascertain a positive correlation. In a study on 64 APS patients and 57 children born to mothers with systemic autoimmune diseases, Andreoli et al. showed a high prevalence of anti-DI in APS while there was a low anti-DI frequency reported in anti-β2GPI positive healthy children.

A direct demonstration of the pathogenic effect of anti-DI antibodies has been recently shown using a human monoclonal IgG (MBB2), the infusion of which brought about fetal losses in pregnant mice and blood clots in rat mesenteric microcirculation following priming with lipopolysaccharide (LPS). Interestingly, a variant of this antibody, lacking the CH2 domain (MBB2DACH2), was effective in preventing blood clot formation and fetal loss induced by aPL. A recombinant human domain I has also been shown to inhibit the ability of polyclonal human IgG from a patient with APS to cause thrombosis or to enhance tissue factor activity in an animal model. Using polyclonal IgG from patients with APS, anti-domain I-rich IgG significantly enhanced prothrombotic ability in vivo compared with anti-domain I-poor or NHS-IgG, suggesting that the ability of human APS-derived IgG to cause thrombosis in mice is concentrated in the anti-domain I-rich fraction.

A novel approach for developing therapy for APS has shown that tolerogenic dendritic cells specific for domain-I of the β2GPI molecule may have potential in attenuating experimental APS in a murine model, via acceleration of the differentiation of CD4+ T cells to Treg cells, decreased proinflammatory cytokine production, and increased anti-inflammatory cytokine expression (IL-10 and TGFβ).

Antibodies to prothrombin
Prothrombin (factor II) is an important antigenic target for aPL in APS. Prothrombin is a vitamin K-dependent single-chain glycoprotein of 579 amino acid residues with a molecular weight of
72-kDa. It circulates in normal plasma at a concentration of approximately 100 μg/ml\textsuperscript{29}. Antibodies directed to human prothrombin (aPT) and the complex of phosphatidylserine/prothrombin (APS/PT) are detected by ELISA and have been strongly associated with APS\textsuperscript{30}. While the presence of these antibodies have been shown to correlate in some cases\textsuperscript{31}, it seems that aPT and APS/PT belong to different populations of autoantibodies\textsuperscript{32}.

A systematic review of the literature including 6000 patients and 1400 controls has been recently reported\textsuperscript{33}. APS/PT was shown to represent a stronger risk factor for thrombosis, both arterial and/or venous, than aPT, with an odds ratio (OR) of 5\textsuperscript{34}. Data from our group and others suggest that the risk of thrombosis progressively increases with the increase in number of positive aPL tests\textsuperscript{35-37}. Recently, we showed that testing positive for all three antibodies—LA, anti-β2GPI and APS/PT—was the best diagnostic indication of APS\textsuperscript{38}. In addition, when compared with double or single positivity, this triple combination showed a stronger correlation with clinical events (thrombosis and/or pregnancy loss).

The mechanisms underlying the procoagulant properties of antibodies to prothrombin are not known; currently two are being postulated: a) indirect; through humoral regulators of coagulation (i.e. prothrombin) or b) direct; engaging/activating cell receptors. An isolated report suggests that polyclonal antibodies from patients with anti-protrombin antibodies might act on a ‘target’ molecule expressed at the endothelial cell surface\textsuperscript{39}, although this is as yet uncharacterised. Tissue factor production induced by aPS/PT in procoagulant cells is reported to occur predominantly via activation of the p38 mitogen-activated protein kinase (MAPK) pathway\textsuperscript{40}, similar to the mechanisms implicated in anti-β2GPI-induced cell activation\textsuperscript{41}. In the mouse, active immunisation with prothrombin is associated with increased thrombosis, supporting a role for antibodies to prothrombin in thrombus formation\textsuperscript{42}. In addition, mice treated with IS6 (a mouse monoclonal antiprothrombin antibody) show thrombi that are larger and persist longer than in mice injected with control antibody\textsuperscript{43}.

Pathogenic mechanisms of aPL

Despite our incomplete understanding of APS pathogenesis, the major facets have been defined in recent years. Thrombosis, a key feature of the disease, can be the result of various mechanisms, including endothelial cells, monocytes, platelets, coagulation, and complement pathways, as well as blocking of the fibrinolytic and anticoagulation pathways. The conventional understanding is that aPL antibodies bind to receptors on target cells, causing their activation and leading to thrombosis in large vessels\textsuperscript{44}. A number of processes have been implicated as effectors of a prothrombotic state in APS. These include: the generation of tissue factor\textsuperscript{45,46}; complement activation\textsuperscript{47-49}; activated platelet-enhanced endothelial activation\textsuperscript{50-52}; monocye protease receptor activation\textsuperscript{53}; and the generation of DNA nets by neutrophils\textsuperscript{54,55}.

aPL have been proposed to bind to cellular membranes via various different receptors, including annexin A2\textsuperscript{56-58}, apolipoprotein E receptor 2 (ApoER2)\textsuperscript{59-61}, low-density-lipoprotein receptor (LDL-R)\textsuperscript{62}, megalin\textsuperscript{63}, Toll-like receptors 2\textsuperscript{64-66} and 4\textsuperscript{67,68}, and the very-LDL-R and P-selectin glycoprotein (GP) ligand-1. It has also been shown that β2GPI is able to directly bind to the platelet adhesive receptor GPIb\textsuperscript{69,70} and the platelet factor4 (PF4)\textsuperscript{71}.

Antibody binding to aPL receptors on target cells activates intracellular mediators like nuclear factor kappa B (NF-κB) and p38MAPK\textsuperscript{72}. aPL have also been shown to activate the phosphatidylinositol 3-kinase (PI3K)–AKT pathway. Activation of this signaling cascade engages the mammalian target of rapamycin (mTOR), a kinase modulating cellular growth, proliferation and survival\textsuperscript{73}. Polyconal aPL from APS patients induced a marked increase in S6RP and AKT (Ser473) phosphorylation, two of the components of the mTOR pathway, mediating intimal hyperplasia and chronic vasculopathy often seen in APS\textsuperscript{74}.

aPL and the Coagulation System

aPL have been reported to inhibit the anticoagulant properties of activated protein C (APC)\textsuperscript{75,76}, impair fibrinolysis\textsuperscript{77-79}, reduce tissue factor pathway inhibitor (TFPI) activity\textsuperscript{80-82} and β2GPI-thrombin interaction\textsuperscript{83-85}, and disrupt the annexin A5 anticoagulant shield\textsuperscript{86-88}. The binding of aPL to β2GPI diminishes β2GPI complement regulatory function with the consequent impaired clearance of apoptotic cells\textsuperscript{89}.

aPL as risk factors for thrombosis: Scoring Systems in APS

One of the unexplained matters in APS is why some patients develop thrombolic events while others present with morbidity in pregnancy. While a minority of patients may also develop a life-threatening “catastrophic” form of APS with multiple organ involvement and a high death rate, others never develop any aPL-related manifestation.

In this context, assessing the patient risk of developing an aPL-related manifestation is crucially important for physicians. Three score systems have been formulated to quantify the risk of thrombosis/obstetric events in APS\textsuperscript{90-92}.

In 2011, a risk model for APS diagnosis was developed based on patient positivity for aPL along with their titre and the results obtained for LA investigation\textsuperscript{93}. Probability estimates for diagnosis of APS were obtained using logistic regression equations and the authors demonstrated that multiple aPL positivity, primarily the triple association of LA, aCL and anti-β2GPI, increased the risk of APS. LA was shown to be the strongest aPL associated with the diagnosis of APS.

In an attempt to quantify the risk based on the aPL profile, Otomo et al.\textsuperscript{94} designed the “antiphospholipid score” or aPL-S. The aPL profiles were analyzed using six ELISAs (IgG/IgM aCL, IgG/IgM anti-β2GPI, and IgG/IgM aPS/PT) and five clotting assays for LA. An algorithm generated this score, with each assay being assigned different points weighted on the relative risk of having a
clinical manifestation of APS. The prevalence of APS manifesta-
tions increased with the increasing aPL-S, suggesting that the
aPL-S could serve as a marker of the “probability” of APS and a
valuable tool for predicting thrombosis. An independent validation
in a separate cohort of 211 consecutive SLE patients confirmed the
aPL-S correlation with a history of thrombosis or pregnancy loss81.

Our newly developed alternative score for APS diagnosis (Global
APS score or GAPSS) is based on independent thrombosis and
pregnancy loss risk factors85. This score accounts for established
cardiovascular risk factors and the autoimmune antibodies profile
in addition to the aPL profile (criteria aPL and non-criteria aPL85).
We developed and validated the score system in a SLE cohort. The
analysis included data on clinical manifestations, conventional
cardiovascular risk factors, aPL and autoimmune profile (including
ANA, ENA and anti-dsDNA, among others). Weighted points
proportional to the β-regression-coefficient values were assigned
to each independent risk factor identified by multivariate analysis.
Validation was performed in a second cohort of patients showing
statistically significant higher values of GAPSS in those with a
clinical history of thrombosis and/or pregnancy loss when
compared to those without events.

When applied in a prospectively followed-up cohort of SLE
patients, an increase in the GAPSS during this follow up was
found to be associated with a 12-fold increase in the risk of vas-
cular events. In detail, an increase of more than 3 GAPSS points
seemed to have the best risk accuracy for vascular events with a
hazard ratio of 4885.

This score was also applied to a cohort of primary APS, higher
values of GAPSS were seen in APS patients who experienced
thrombosis when compared to those with previous pregnancy
loss alone. In addition, GAPSS was able to discriminate patients
who experienced recurrent thrombotic events from those without
recurrences84.

This score was independently validated by two groups. Zuily et al.85
evaluated the validity of the GAPPS to predict thrombosis in a
prospective multicentre cohort study. GAPPS values were
significantly higher in patients who experienced a thrombotic event
when compared to those without a reported GAPPS above
16 as a significant predictor of thrombosis in this population. Oku
et al.86 confirmed that GAPPS can be successfully used to
quantify risk in an independent cohort of patients with autoim-
mune diseases. GAPSS correlated with a history of APS symptoms,
particularly with thrombosis, implying it can be used as an
appropriate quantitative marker for APS.

Classification vs. diagnostic criteria
As stated above, in 1999, definitive classification criteria for APS
were published in an international consensus statement8 and a
subsequent revision was made in 20068. A patient with APS must
meet at least one of two clinical criteria (vascular thrombosis or
complications of pregnancy) and at least one of two laboratory
criteria including the persistent presence of lupus anticoagulant
(LA), anticardiolipin antibodies (aCL) and/or anti-β2GPI anti-
obodies of IgG or IgM isotype at medium to high titres in patient’s
plasma.

These classification criteria are aimed at identifying well defined,
relatively homogeneous group of patients, all sharing key features
of the condition, as they do not reflect the different features of
the disease, as diagnostic criteria should81. To date, there are no
diagnostic criteria available for APS and, therefore, even with a
lack of ‘essential’ or ‘key’ features, clinicians should be encour-
ged to consider the diagnosis in the presence of ‘minor’ features,
providing other causes have been ruled out.

aPL carriers
Overall data from available studies suggest that asymptomatic
aPL carriers bear a 0–2.8% annual risk of developing a thrombotic
event86. While the presence of aPL is necessary but not sufficient to
provoke a thrombotic event, the “second hit” hypothesis suggests
that an additional trigger is needed to initiate a vascular event in
aPL carriers.

An early study from 199887 evaluated the prevalence of thrombo-
sis in aCL positive patients with SLE. The authors reported that
52% of aCL carriers developed a thrombotic event during the
10-year follow up, opening the question on the importance of these
antibodies as risk factors for thrombosis. From then, few other
studies have estimated the incidence of thrombosis in asympto-
matic carriers with aPL. A total of 178 asymptomatic aPL carri-
ers without underlying autoimmune diseases underwent a 3-year
prospective observational cohort study and no thrombotic events
were reported during follow up86. The APLAS study, a ran-
domized, double-blind, placebo-controlled trial investigating
the efficacy of low-dose aspirin (LDA) as primary prevention
of thrombotic events showed a low incidence of thrombosis in aPL
carriers, events occurring in all but one of the cases, in the presence
of concomitant thrombosis risk factors and/or systemic autoim-
mune disease at the time of thrombosis87. A prospective study
identified hypertension and LA as independent risk factors for a
first thrombotic event in asymptomatic aPL carriers88.

A recent study evaluating the efficacy and safety of LDA vs. LDA
plus low-intensity warfarin in the primary thrombosis prevention
of aPL-positive patients with SLE and/or obstetric morbid-
ity reported an incidence of 1.8 events/100 person-years in the
randomized group89. Interestingly, this incidence was increased to
4.9 events/100 person-years in the observational arm with hyper-
tension being the most frequent additional risk factor.

Evidence shows that patients with more than one positive test, and
particularly those with all three positive aPL tests (referred to as
triple positive), are those with a strong association with clinical
events90,91. Therefore, aPL carriers should be risk-stratified
according to the aPL status, the presence of other cardiovascu-
lar risk factors that should be closely monitored and controlled
whenever possible, and the concomitance of other systemic
autoimmune diseases.
Conclusions
Studies are underway to establish the value of testing for new aPL specificities in the identification of APS in patients with thrombosis and/or pregnancy morbidity, particularly in those for whom repeated testing produces negative results with currently available methods. While their clinical importance and mechanisms of action are far from being fully explored, available data suggest that the presence of these other aPL, particularly anti-DI and aPS/PT antibodies, are useful for risk stratification.

Ongoing research focuses on cell receptors and intracellular signaling pathways involved in the cell activation mediated by aPL. The clarification of these mechanisms is crucial to a better understanding of pathogenesis of APS. Although some controversial data still exist in regards to new specificities, most of the available reports support the association between aPS/PT, and to a lesser extent anti-DI, and the clinical manifestations of APS.

Additional studies to conclusively define the relevance and prognostic impact of testing for these antibodies in the daily routine clinical practice are still required.

When assessing risk, the use of GAPSS may provide valuable information regarding thrombosis or pregnancy loss risk, switching from the concept of aPL as simply diagnostic antibodies to aPL as relevant risk factors for clinical events.

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