

Update on antiphospholipid antibodies: clinical significance

Antiphospholipid antibodies (aPL) are autoantibodies directed to phospholipid-binding proteins and classically related to thrombosis and pregnancy loss in the so-called antiphospholipid syndrome (APS). A wide spectrum of clinical manifestations have been observed in individuals with aPL. These may be asymptomatic, or they may exhibit clinical features suggesting APS, but not fulfilling the International Classification Criteria; typical APS manifestation in the absence of detectable aPL is defined as seronegative APS; microangiopathic APS may involve either one tissue only or may present with widespread thrombosis episodes accompanied by acute multi-organ failure in the catastrophic syndrome. The detection of different aPL profiles seems to increase the diagnostic specificity of the tests. The future research should focus on the development of assays that selectively identify aPL associated with increased risk of thrombosis or pregnancy complications, in order to lead to therapeutic strategies able to prevent unresolved problems associated with aPL.

KEYWORDS: antiphospholipid antibodies ■ antiphospholipid syndrome ■ prophylaxis ■ treatment

Antiphospholipid antibodies (aPL) represent a heterogeneous group of antibodies that recognize various phospholipids, phospholipid-binding proteins and phospholipid-protein complexes. The antigenic targets include β 2-glycoprotein (β 2GPI) [1], protrombin (PT) [2], activated protein C [3], tissue plasminogen activator, plasmin [4] and annexin A2 [5].

As many of the targeted proteins are involved in the process of coagulation and its regulation, it follows that these antibodies may interfere with homeostasis and eventually contribute to the occurrence of thrombotic events.

The most commonly used tests to detect aPL are lupus anticoagulant (LA), a functional coagulation assay, and anticardiolipin antibody (aCL) and anti- β 2-glycoprotein-I antibody (a β 2GPI), which are enzyme-linked immunoassay (ELISAs).

Some aPL can be found in patients with autoimmune diseases. Among patients with systemic lupus erythematosus (SLE), the reported prevalence of aPL ranges from 12 to 34% [6]. aPL can also appear during the course of infectious diseases, simultaneously to cancer, or can be drug-related; they can also be present as an isolated phenomenon in healthy individuals [7]. In a young healthy population, the occurrence of both LA and aCL is approximately 1–5%; the prevalence increases with age, especially in elderly individuals suffering from chronic disease [8]. Such a prevalence in the healthy

population may be due to the cut-off values used for aPL assays, which are usually calculated as a 95th or 99th percentile distribution of healthy subjects. However, the presence of circulating aPL in healthy subjects may be alternatively interpreted from a biological point of view, supporting the hypothesis that pathological aPL may derive from naturally occurring (nonpathogenetic) autoantibodies as the consequence of a break in tolerance against self components. In particular, experimental data suggest that a molecular mimicry between microbial antigens and the β 2GPI molecule may be responsible for the break of tolerance and the auto-antibody production. Anti- β 2GPI antibodies react with the molecule complexed with coagulation factors and with the molecules expressed on the cell membrane of different cell types. In the former case it has been suggested that the antibodies may interfere with natural anticoagulant and fibrinolytic systems by inducing a procoagulant state. When bound to β 2GPI on the cell membranes, the antibodies may activate endothelial cells, monocytes and platelets, eventually favoring a pro-coagulant phenotype [9].

The pathogenicity of aPL has been extensively studied. Some of the mechanisms causing thrombotic events include:

- Inhibition of activated protein C (APC) [10];
- Promotion of platelet activation [11];

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- Interaction with endothelial cells (ECs) and induction of adhesion molecule expression and monocyte adhesion [12];
- Antithrombin inhibition [13];
- Activation of the complement pathway [14];
- Inhibitory binding to hemostasis and fibrinolytic proteases such as APC [15] and plasmin [16].

On the other hand, aPL are a well-known cause of pregnancy morbidity. Several pathogenetic mechanisms have been suggested to play a role in these obstetric manifestations:

- Induction of intraplacental thrombosis (by disruption of the annexin A5 crystal shield [17]);
- Impairment of physiological placentation (by direct cellular injury, apoptosis, inhibition of proliferation and syncytia formation, decreased human chorionic gonadotrophin production and defective invasiveness) [9];
- Local inflammatory events including complement activation [9].

The aim of this review is to identify the main clinical manifestations associated with the presence of aPL.

Antiphospholipid profiles

Lupus anticoagulant, aCL and a β 2GPI assays are included in the revised formal classification criteria of antiphospholipid syndrome (APS) and it is stated that patients should be stratified according to the different positivity profiles [18]. In fact the interpretation of aPL laboratory profiles is important to assess the risk of a single patient, and it also helps to perform a correct diagnosis and to establish the required treatment. In particular, clinicians should focus on the titer and the isotype of aPL, and on the presence of multiple positive tests for aPL, as it seems that risk may vary upon these factors [19]. However, this process is limited by the number of difficulties still connected to the standardization of laboratory assays [20].

In a meta-analysis study, LA persistent positivity was found to be the strongest risk factor for thrombosis occurrence. On the other hand, it was observed that patients positive for LA on two or more occasions at least 12 weeks apart can be considered at low risk of thrombosis in the absence of other traditional risk factors. [21].

It is even more difficult to interpret an isolated persistent aCL or a β 2GPI positivity, whose prevalence may vary among different studies. It is estimated that 3–10% of patients with APS have a β 2GPI as the only positive test [18].

Anticardiolipin ELISA is generally considered to have high sensitivity but low specificity. Such an assay is able to mostly recognize antibodies against the cardiolipin– β 2GPI complex (β 2GPI-dependent aCL). However, aCL that bind directly to cardiolipin, independently from plasma proteins, can also be detected. Such antibodies are generally induced by certain drugs and infectious diseases, and they are rarely related to a prothrombotic phenotype [22]. Therefore, it must be kept in mind that aCL ELISA may be aspecific due to the presence of infection-related antibodies; such false-positive results may be misleading for the establishment of a correct APS diagnosis.

Only the IgG isotype was shown to be associated with the presence of previous thromboembolic events or obstetric complications [23], and to be a significantly higher risk factor for cerebral stroke and myocardial infarction than for deep-vein thrombosis (DVT) [24].

However, the antibody titer was reported to be critical. In fact, according to the revised International Classification Criteria for definite APS [18], the threshold for significant positive aCL was established at a value of 40 G phospholipid or M phospholipid units or more, while the cut-off value was suggested as the 99th percentile or more.

There is evidence that a β 2GPI antibodies are an independent risk factor for thrombosis and pregnancy loss [25,26]. It is important to underline that antibodies directed to domain I of β 2GPI express LA activity and correlate strongly with thromboembolic events [27]. On the other hand, a β 2GPI against domain IV have been described in nonthrombotic conditions [28]. Therefore, it seems that different subpopulations of a β 2GPI carry different degrees of pathogenic potential. Some autoantibodies to β 2GPI may not be pathogenic, and this might explain why studies on their detection have not produced uniform results [29].

The Standardization Group of the European Forum on aPL tried to homogenize a β 2GPI test results from various laboratories to provide results in common units [30]. Nowadays, the introduction of monoclonal a β 2GPI antibodies as common international calibrators should solve the problem of test reporting [18].

The simultaneous positivity of aCL and a β 2GPI of the same isotype is very helpful, as it excludes the presence of infective antibodies and confirms the presence of relevant autoimmune antibodies. This aPL profile (IgG isotype for both tests) is associated with thrombosis, but

even more so with pregnancy morbidity [23]. A full positive pattern appears to reflect the presence of significant amounts of autoantibodies to human β 2GPI, with a consequent increased risk of thrombosis-related events and/or obstetric complications [31].

Finally, it is worth mentioning aPL of the IgA isotype, both aCL and a β 2GPI. They were not included as a laboratory criterion in the last consensus statement due to the lack of evidence suggesting their utility in increasing the diagnostic power [18]. However, some studies suggest that aCL IgA may be useful in assessing those patients with high clinical suspicion of APS, but negative results on IgG and IgM assays [19].

Clinical features

A broad spectrum of clinical manifestations is associated with aPL. Frequently we observe an association between aPL and classical clinical manifestations such as venous and arterial thrombosis and recurrent miscarriage, which characterize the so-called APS, but asymptomatic aPL-positive patients do exist. In addition, as quoted above, aPL may occur as a result of certain drugs or infections [22]. aPL during infectious diseases are usually of the IgM isotype, and their positivity is only temporary. These antibodies are not usually associated with clinical complications [32]. However, the link between APS and infections is more complex, and will be discussed later on.

■ Asymptomatic subjects

It still remains poorly understood why, among individuals with aPL, some develop thrombosis and some do not, and why asymptomatic carriers display clinical symptoms after many years. A 'two-hit hypothesis' has been suggested to explain the observation that clinical events occur only occasionally in spite of the persistent presence of aPL. aPL may represent a predisposing risk factor (first hit), but the addition of a triggering risk factor (second hit) may be required for thrombosis development [9,33]. Identifiable risk factors for thrombosis include immobility, surgery, hypertension, atherosclerosis, elevated low-density lipoprotein or high-density lipoprotein cholesterol, obesity, smoking, malignancy, diabetes mellitus, pregnancy, older age, use of oral contraceptive pill, congenital thrombophilic factors such as mutations of Factor V Leiden, and protein C and S deficiency, among others. In particular, infections are claimed to be an important second hit, which is able to affect morbidity and mortality in patients with

persistent aPL positivity. Moreover, infections are reported to be a trigger factor for a severe form of APS, the catastrophic one, which will be described in detail later on.

Recently, the genetic background has also been proposed for explaining why positive carriers develop clinical manifestations. Some authors suggest that some *HLA* alleles carry the risk to produce pathogenic aPL, and this is independent from other thrombogenic risk factor. In fact, they demonstrated an association between aCL and *HLA-DR4*, *-DR7*, *-DRw53* and *-DQB1*0302* in primary APS [34]. It was also reported that the valine/leucine 247 polymorphism of β 2GPI was associated with both the presence of a β 2GPI and a stronger reactivity with such antibodies in comparison with the wild-type allele [35]. In addition, other authors have proposed that aPL manifestations are associated with the polymorphism of genes encoding for signaling pathways of proinflammatory mediators. For instance, a polymorphism of the *Fc γ R1IA* gene was found to be associated with APS [36]; on the other hand, a polymorphism of *TLR4* was described to be protective against thrombosis, by downregulation of the inflammatory response at the endothelial level [37].

The asymptomatic aPL carriers require a meticulous assessment in relation to the above-mentioned thrombophilic conditions and a primary prophylaxis of the reversible risk factors. In fact, it is reasonable to assume that in asymptomatic subjects with aPL the likelihood of developing a thrombotic event increases much more in the presence of additional risk factor for venous or arterial thrombosis [6]. At present, evidence-based recommendations for the prophylactic treatment of asymptomatic aPL carriers are lacking. In a recent randomized, double-blind, placebo-controlled trial, low-dose aspirin appeared to be no better than placebo in preventing first thrombotic episodes in persistently asymptomatic aPL-positive patients [38]. However, a recent multicenter retrospective follow-up study [39] and the guidelines based on the Framingham Heart Study [40] suggest that a primary thrombosis prevention strategy should be risk stratified and determined taking into account age, traditional cardiovascular risk factors, systemic autoimmune disease and the aPL antibody profile. This approach can help the physicians in the everyday decision-making.

With regards to pregnancy, maternal aPL increases the risk of fetal loss and premature birth [41]. It is generally believed that low-dose

aspirin treatment may significantly improve both maternal and neonatal outcomes, even if no trial has demonstrated a significant benefit of this treatment at present [42]. On the other hand, large clinical trials have demonstrated that low-dose aspirin is relatively safe, and consequently justify its administration as prophylaxis during pregnancy.

Regarding the maternal side, the risk for thrombosis is increased even during physiological pregnancy, being estimated to be up to three- to five-fold higher than in the general population [43]. Post-partum, linked to important hormonal changes and blood volume restriction, is recognized to be a period at increased thrombosis risk in normal women also [43]. In asymptomatic aPL-positive patients, special caution should be taken in the post-partum period due to the high maternal risk of thrombosis. In fact, during puerperium, the first thrombotic episodes in patients with aPL are not rare [44]. Thus, aspirin is not always considered sufficient thromboprophylaxis, and low-molecular-weight heparin can be used up to 6 weeks after delivery [45]. However, the prophylactic management of pregnant women with aPL should be tailored case by case, taking into account any maternal risk factors, such as current organ damage (cardiac, cerebral, pulmonary and renal), maternal age and the coexistence of clinically evident systemic autoimmune disease, such as SLE [44].

■ Antiphospholipid syndrome

The revised International Classification Criteria for Definite Antiphospholipid Syndrome established that APS diagnosis requires the combination of at least one clinical and one laboratory criterion. The clinical criteria include recurrent thrombosis (arterial, venous or small-vessel) occurring in any tissue or organ and/or pregnancy morbidity (Box 1). Laboratory criteria require the positivity of at least one of the three tests: LA, aCL (IgG/IgM) and/or a β 2GPI (IgG/IgM) at high titers; positive values need to be confirmed on repeat testing 12 weeks apart [18] (detailed in Box 1).

In patients with autoimmune disorders (SLE, rheumatoid arthritis and so on) it is possible to find aPL associated with typical APS clinical manifestations. The episodes of thrombosis are similar in 'primary APS' (without other connective tissue disease) and 'secondary APS' (with connective tissue disease). The revised International Consensus Statement [18] eliminated the primary versus secondary distinction, because there are not differences in the clinical consequences of aPL among patients in these two categories [46]. The new proposal is that patients with primary APS should be described as simply having APS, and the term secondary APS be replaced with APS and the specific mention of the autoimmune disorder with which it is specifically known to be associated (i.e., APS and SLE) [18].

Box 1. International Classification Criteria for definite antiphospholipid syndrome.

Clinical criteria

- Vascular thrombosis
 - One or more clinical episodes of arterial, venous, or small-vessel thrombosis, with the exception of superficial venous thrombosis, in any tissue or organ. Thrombosis must be confirmed using imaging or Doppler studies or histopathology. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
- Pregnancy morbidity
 - One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation.
 - One or more premature birth (<34 weeks of gestation) of morphologically normal neonate, because of eclampsia, severe pre-eclampsia and placental insufficiency.
 - Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation (excluded maternal anatomic or hormonal abnormalities and chromosomal cause).

Laboratory criteria

- LA detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on LAs/ phospholipid-dependent antibodies) is considered positive if present in plasma.
- Anticardiolipin antibodies of IgG and/or IgM isotype measured by a standardized ELISA are considered positive if present in serum or plasma, in medium or high titer (i.e., >40 GPL or MPL, or greater than the 99th percentile).
- Anti- β_2 glycoprotein-I antibodies (a β 2GPI) of IgG and/or IgM isotype measured by a standardized ELISA, according to recommended procedures, are considered positive if present in serum or plasma, in titer greater than the 99th percentile, on two or more occasions, at least 12 weeks apart.
- Each test should be confirmed to be positive on two or more occasions, at least 12 weeks apart.

ELISA: Enzyme-linked immunoassorbent assay; GPL: G phospholipid; LA: Lupus anticoagulant; MPL: M phospholipid.
Adapted and modified from [18].

The current recommendation for secondary thrombosis prevention in APS patients is life-long warfarin. The necessity, duration and intensity of warfarin treatment is still debated.

In the general population, patients who develop thrombosis because of an acquired risk factor display a low risk for recurrences following the discontinuation of anticoagulants. It should be reasonable that aPL-positive patients who develop an event triggered by an acquired, reversible thrombotic risk factor can be taken off anticoagulation therapy, but APS *per se* seems to have an increased risk of recurrent thrombosis if anticoagulation therapy is stopped [47]. This makes discontinuation of anticoagulation treatment difficult. The APS management for secondary prophylaxis of venous thrombosis is anticoagulation of moderate intensity (international normalized ratio [INR] 2–3) [48]. Some prospective randomized trials suggest that both moderate- (INR 2–3) and high-intensity (INR 3–4) anticoagulation are similarly protective in these patients after the first venous thrombosis [49,50]. However, high-intensity anticoagulation is linked to a higher number of bleeding episodes. A recent systematic review of secondary thromboprophylaxis in patients with aPL underlines that untreated patients had high recurrence rates (19–29% per year), and it demonstrates an evident a dose effect of oral anticoagulant, with fewer thrombotic events among patients treated with high-intensity anticoagulation (INR of 3–4) as compared with those treated with low-intensity (INR of 2–3). These conclusions are limited by the retrospective nature of the study, and at present high-intensity anticoagulation (INR of 3–4) for APS patients is considered only in the presence of previous arterial events or recurrent venous thrombosis despite moderate-intensity anticoagulation [48]. Before increasing the warfarin dose, the identification and reduction of possible non-aPL risk factors for thrombosis is strongly suggested, while after high-intensity anticoagulation failure, low-dose aspirin, or hydroxychloroquine and/or statins can be added to treatment [48]. However, it is recommended that the specific therapy is tailored on an individual basis, taking into account the severity of the initial thrombotic event, the concurrent presence of other vascular risk factors or thromboses recurrence and the estimated bleeding risk according to age, bleeding history and concomitant drugs. As a matter of fact, in the case of stroke, anticoagulation is not the only treatment claimed to be adequate as secondary prophylaxis in APS patients. Some reports

actually suggest that aspirin can be effective as well [51]. Therefore, clinicians should evaluate every patient individually and choose the treatment that best combines efficacy and safety for that particular case.

In addition, for patients with formal APS diagnosis, the ideal treatment during pregnancy would improve maternal and fetal–neonatal outcome by preventing pregnancy loss, pre-eclampsia, placental insufficiency, and preterm birth, and reducing or eliminating the maternal thrombotic risk [52]. Actually, a prophylactic dose of heparin is used in APS patients with a history of pregnancy morbidity, although its efficacy has not been proved in a recent meta-analysis [53]. Instead, therapeutic-dose heparin is used in APS patients with previous thrombotic events. Based on the few controlled trials available, in both the previously described situations low-dose aspirin is generally utilized, even if this could still be debated [44].

The pregnancy of women with previous thrombosis is obviously different from that of patients with gestational complications only [54]. Patients with previous thromboses and APS usually receive lifelong anticoagulation with warfarin. During the pregnancy, women must discontinue warfarin because of its teratogenic effect. The optimum time to switch such women to heparin is debated. Currently, the prevalent opinion is to advise patients to conduct a pregnancy test very early at the first missed period, and if the pregnancy test is positive, to promptly switch from warfarin to heparin [55]. Nevertheless, for patients with particularly severe recurrent thromboses, such as stroke, warfarin can be restarted, but only after 14 weeks of gestation when organogenesis is complete [55]. Because of possible bleeding, warfarin should be stopped before delivery. Since the most common complication of APS pregnancies is preterm delivery [56], this therapeutic option should be limited to only those in real need.

In patients failing treatment with aspirin and heparin, the addition of IVIG has been shown to be effective in case reports [57]. In addition, corticosteroids, hydroxychloroquine, plasmaferesis and increase of heparin dose are strategies used by physicians [48].

■ Microangiopathic antiphospholipid syndrome

Thrombotic thrombocytopenic purpura (TTP), hemolysis, elevated liver enzymes and low platelets syndrome (HELLP), hemolytic–uremic syndrome (HUS) and thrombotic microangiopathic

hemolytic anemia (TMHA) have all been reported to be associated with aPL. These diseases are commonly characterized by microvascular occlusive disease and, along with the catastrophic antiphospholipid syndrome (CAPS), have been recently grouped under the name of microangiopathic antiphospholipid-associated syndromes (MAPS) [58]. This microvascular occlusion might be part of the APS, and should be distinguished from other conditions where the aPL appear not to be pathogenic, but to be a consequence of small-vessel endothelial damage [58].

Catastrophic antiphospholipid syndrome is a rare and distinct form of APS that results in widespread, repeated and sudden thrombotic episodes, accompanied by acute multi-organ failure, systemic inflammatory response and thrombotic microangiopathy [59]. Clinical features are the consequence of organ and tissue ischemia, including cerebral injury, myocardial, renal and acute respiratory failure. A collection of clinical and laboratory features, treatment and outcome of CAPS is provided by the International CAPS Registry started in 2000 by Richard Cervera and the late Ron Asherson. Today, the registry includes more than 300 cases of CAPS, and allows free consultation online by the physicians [60]. CAPS classification criteria and treatment were proposed by the 2002 International Taormina Consensus Statement on Classification and Treatment of CAPS. For the diagnosis, it is necessary to demonstrate the involvement of at least three organs, systems and/or tissues, along with the presence of aPL. The clinical manifestations have to develop simultaneously or in less than 1 week, and small-vessel occlusion in at least one organ or tissue must be confirmed by histopathology [61]. Although infections, surgical procedures, trauma, SLE flares, drugs or oral contraceptive are frequently recognized as trigger factors, in approximately 30% of patients no precipitating factors are identified.

Acute management of CAPS requires an early diagnosis. As soon as the diagnosis of CAPS is suspected, it is advised to use an aggressive approach with combination therapy consisting of

anticoagulation, corticosteroids and intravenous immunoglobulin or plasma exchange [59]. In case of nonresponding patients, an additional drug, such as cyclophosphamide or rituximab [62], is generally used. Despite the aggressive approach, approximately half of patients do not survive [60].

HELLP syndrome occurs in less than 1% of normal pregnancies and 10–15% of pre-eclamptic patients [63]. In the general population, HELLP syndrome usually develops between 24 and 32 weeks, it resolves with delivery [64] and the patients rarely develop serious liver complications. Its recurrence is estimated to be approximately 2–6% [65]. In aPL-positive patients, the HELLP syndrome occurs during the second trimester of pregnancy, along with the consequent high rate of fetal deaths. Almost a third of patients develop hepatic infarcts, and aggressive treatment is needed because the majority of the women develop thrombotic complications, but rarely catastrophic APS [66]. Given that in aPL-positive patients HELLP syndrome can be life-threatening and the risk of recurrence is unknown, a future pregnancy should be carefully considered.

■ aPL-associated diseases

Some patients with aPL may display nonthrombotic clinical manifestations, such as livedo reticularis, thrombocytopenia, chorea and hemolytic anemia, aPL-associated nephropathy and heart valve disease. Such aPL-associated diseases have been classified as noncriteria clinical manifestations by the International Consensus Statement (Box 2) [18].

Cardiac abnormalities associated with aPL include valve abnormalities (as stenosis and regurgitation), occlusive arterial disease, intracardiac thrombi, ventricular dysfunction and pulmonary hypertension.

Livedo reticularis is defined as a persistent violaceous, reticular or mottled pattern of the skin of the trunk, arms or legs. From a histological point of view, it is characterized by partial or complete occlusion of the lumen of small- to medium-sized arteries and/or arterioles at the dermis–subcutis border; no evidence of perivascular inflammatory infiltrate and negative direct immunofluorescence examination are also required.

Thrombocytopenia is characterized by platelet count under $100 \times 10^9/l$. It can be subdivided as moderate (platelet count $50\text{--}100 \times 10^9/l$) or severe (platelet count $< 50 \times 10^9/l$).

Antiphospholipid-associated nephropathies include thrombotic microangiopathy involving both arterioles and glomerular capillaries and/or

Box 2. Noncriteria clinical manifestations associated with antiphospholipid antibodies.

- aPL-associated cardiac valve disease.
- aPL-associated livedo reticularis.
- aPL-associated thrombocytopenia.
- aPL-associated nephropathy.
- aPL-associated CNS involvement.

aPL: Antiphospholipid antibodies.

one or more of the following manifestations: fibrous intimal hyperplasia involving organized thrombi with or without recanalization, fibrous and/or fibrocellular occlusions of arteries and arterioles, focal cortical atrophy and tubular thyroidization (large zones of atrophic tubules containing eosinophilic casts). It is also necessary to rule out the presence of vasculitis, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, malignant hypertension and other reasons for chronic renal ischemia. In the case of concomitant SLE, the lesions should be distinguished from those associated with lupus nephropathy.

Neurological manifestations may be heterogeneous and include seizures, migraine, chorea, cognitive dysfunction and multiple sclerosis-like disease. Data on the association with aPL are still contradictory, and little is known about the impact of aPL on the clinical course of such diseases.

Currently, most of the noncriteria manifestations are generally managed with low-dose aspirin, although no available data of literature support this approach. Corticosteroids and/or intravenous immunoglobulin are the first-line treatment for platelet counts of less than $50 \times 10^9/l$. Case reports have documented the effectiveness of rituximab in aPL-related thrombocytopenia [67], but no controlled trials exist. Further studies are necessary to clarify whether anticoagulant treatment is needed to prevent vascular thrombosis in patients with aPL-associated diseases.

■ Seronegative APS

The term seronegative antiphospholipid syndrome (SNAPS) describes patients with typical clinical manifestation of APS, but who are seronegative for all kinds and isotypes of aPL, including LA, at the time of the clinical event [68]. Several possible explanations have been outlined for aPL seronegativity in APS patients [69]:

- Approximately 20–30% of APS patients are positive only for aCL or LA; thus, their positivity can be missed if both tests are not performed in all cases of suspected APS;
- aCL and LA may transiently fall to undetectable levels due to consumption at the time of the thrombotic event, and thus, if initially negative, both tests should be repeated after resolution of the event;
- Some APS patients may only have antibodies binding phospholipids other than cardiolipin;
- Some patients may have antibody binding cardiolipin, but detected by different tests;

- Classical assays may fail to detect IgA antibodies to $\beta 2$ GPI or cardiolipin, since anti-IgA antibodies are not usually included in the commercial preparations.

This last explanation raises a significant dilemma regarding how many aPL tests a diagnostic laboratory should routinely offer to the clinicians. In fact, the majority of laboratories routinely perform aCL, anti- $\beta 2$ GPI and LA. Only a minority offer different tests such as anti-prothrombin, antiphosphatidylserine, antiphosphatidylethanolamine, anti annexin V and so on. Thus, for a case of SNAPS, the clinician and the laboratory have to make a decision regarding whether to spend time and money to have these additional aPL tests performed, or simply to make a diagnosis of SNAPS.

Conclusion

The classical association of thrombotic episodes or pregnancy losses with the presence of aPL detected with the formal assays does not represent the only APS manifestation. Clinicians should consider that more undefined situations may occur; this is the case of the aPL-associated diseases that may involve different organs. Among them, the so-called MAPS are usually challenging for physicians because of their severe and rapid evolution. Finally, the concept of seronegative APS, suggested by analogy with seronegative rheumatoid arthritis, leaves the clinician alone to make their therapeutic decisions.

Along with these clinical issues, laboratory classification is still under debate, depending on the interpretation of different antibody profiles [70].

Given that a prompt and correct treatment can benefit the patients, it is important that physicians bear in mind the complex picture of APS and all possible related conditions in order to establish an early diagnosis.

Future perspective

Despite current therapy (oral anticoagulants and/or anti-aggregants), approximately 20% of patients with aPL still develop morbidity and mortality [56].

A recent multicenter prospective study of 1000 APS patients [56] demonstrated that 5.3% of them died during a 5-year follow-up period. The most common cause of death was bacterial infection, partially because infections appear as complications in severely ill patients, and also because they represent trigger factors for life-threatening syndromes such as CAPS.

Strokes and transient ischemic attacks represent the second cause of death, and are the most frequent recurrent thrombotic events in APS subjects. These patients usually received oral anticoagulants, at various levels of intensity. Such a therapy seems to be effective for venous thrombosis, but apparently is not good enough for arterial thrombosis. Moreover, during the follow-up period, patients on high-dose anticoagulants often suffered from severe hemorrhages that were also reported among the most frequent causes of death.

The third cause of mortality in APS patients is cancer. Recently, some authors have described a higher incidence of aPL in patients with malignancies [71] (principally hematological malignancies); on the other hand, a high rate of hematological malignancy was observed in the follow-up of a cohort of aPL carriers [72].

The future research agenda should focus on the unsolved problems regarding causes of morbidity

and mortality. A particular effort should be made to avoid/effectively care for infections in APS patients, and to apply specific therapy tailored to the clinical picture and on individual risk. Finally, the connection between aPL and malignancies should be better investigated, both on a research basis and in clinical practice, in order to establish a proper follow-up of patients for early diagnosis of such severe conditions.

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Executive summary

Antiphospholipid antibodies profile

- Isotype, titer and multiple positivity might help to assess the complication risk of a single patient.

Clinical features

- Antiphospholipid antibodies (aPL) are associated with a wide spectrum of clinical manifestations, not limited to recurrent thrombosis and fetal losses.
- Undefined situations may occur in association with aPL, and may require a meticulous assessment for the prevention of complications.
- The genetic background and environmental factors may determine the clinical manifestations.

Conclusion

- It is important that physicians understand the complex pictures associated with aPL in order to avoid their possible severe complications.
- Information on the predictive value of laboratory tests with respect to thrombosis is still limited. Therefore, the development of new assays that selectively identify aPL antibodies associated with an increased risk of thrombosis is necessary in order to help the clinicians to establish the most appropriate therapeutic strategies.

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