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Novel diagnostic and therapeutic frontiers in thrombotic Antiphospholipid Syndrome

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Short Title

New frontiers in APS

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Abstract

Background

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity, associated with a persistent positivity for antiphospholipid antibodies (aPL). The current classification criteria for APS include three laboratory tests: lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2 glycoprotein-I (β2GPI). To date, the therapeutic approach for thrombotic APS mainly centers on long-term anticoagulation with vitamin K antagonist (VKA).

Purpose of the review

APS management may represent a challenge for the treating physicians. Patients with different aPL profiles need a tailored risk-stratified approach. Besides, in patients with recurrent thrombotic events despite therapy with VKA or those with microvascular involvement new therapeutic options are highly needed.

In this review we aim to elucidate recent findings about new aPL specifities, available risk scoring models and novel therapeutic approaches in APS management.

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1. Intro

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis (arterial and/or venous) and/or pregnancy morbidity (miscarriages, fetal deaths, premature births, and late pregnancy complications) associated with a persistent positivity for antiphospholipid antibodies (aPL). The current classification criteria for APS include three laboratory tests: lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2 glycoprotein-I (β2GPI). However, growing evidences are supporting that in some selected cases non-criteria aPL might also play a role. To prevent detection of transient antibodies, tests must be positive ≥ 2 occasions, at least 12 weeks apart.

Herewith, we aim to discuss new insights in APS, including laboratory testing, thrombotic risk assessment and upcoming therapeutic options.

2. Role of new antibodies specificities

In the current clinical practice, aCL, anti-β2GPI antibodies and the LA have been the most established tests for the diagnosis of APS[1]. The clinical utility of aPL assays for autoantibodies other than the routinely used is currently under debate. Indeed, current lines of research are examining the usefulness of testing for new aPL specificities in identifying APS in patients with thrombosis and/or pregnancy morbidity, particularly in those who are repeatedly negative for the criteria aPL. Among the so called extra-criteria aPL tests, anti-phrothrombin (and mainly anti-phophosphatidylserine/prothrombin, aPS/PT) antibodies and anti-β2GPI glycoprotein-I Domain 1 antibodies, have been proposed to potentially improve the diagnostic accuracy and, especially when assessing the risk for both thrombosis and pregnancy morbidities in patients suspected of APS.

2.1 Anti-β2GPI glycoprotein-I Domain 1 antibodies

Although the physiological functions of β2GPI are still uncertain, available evidence supports a pathogenic role for anti-β2GPI antibodies contributing to thrombosis and pregnancy morbidity.

However, not all patients positive for the presence of anti-β2GPI antibodies develop clinical aPL-related manifestations. This heterogeneity in the pathogenic potential of anti-β2GPI antibodies might be ascribed to the molecular structure of β2GPI, presenting multiple antigenic specificities that can be targeted by different autoantibodies. The β2GPI has five homologous domains (D1 to D5) and the main epitope that has been found to be associated with APS involves regions of D1 and growing evidence, both in vivo and in vitro, has resulted in the identification of domain I as the "immunodominant epitope"[2], supporting a role for anti β2GPI-DI antibodies in the development of APS-related clinical manifestations.

Recently, a systematic review and meta-analysis that included a total of 1585 patients, reported an overall estimated median prevalence of anti β2GPI-DI antibodies of 44,3% in patients with APS and/or systemic lupus erythematosus (SLE) and a significant higher prevalence of anti-β2GPI-DI antibodies among APS patients compared to SLE alone[3]. Furthermore, when analyzing the thrombotic risk assessment associated with anti β2GPI-DI antibodies positivity, the study reported an overall OR 1,99; 95% CI 1,52-2,6; p<.0001. Anti-β2GPI-DI antibodies might represent a promising tool when assessing thrombotic risk in APS patients.

2.2 Antiprothrombin and anti-phosphatidylserine/prothrombin antibodies

Antiprothrombin (aPT) and aPS/PT antibodies are directed against negatively charged phospholipids other than cardiolipin. aPT antibodies are commonly detected by ELISA, using prothrombin coated onto irradiated plates, or prothrombin in complex with phosphatidylserine. Although aPT and aPS/PT antibodies can co-exist in the same patients, they are part of two different populations of autoantibodies. Currently, most of the studies in the literature support their role in helping defining APS diagnosis, as well as the association between antiprothrombin antibodies, in particular aPS/PT , and the clinical manifestations of APS[4]. Although existing data is promising, further studies are needed in order to establish the real impact and clinical significance of these antibodies in routine testing.

3. Risk assessment and aPL

When assessing risk for clinical manifestations of APS, aPL titres as well as their single, double or triple presence, have all been suggested to have a different distinct significance[5]. In general, the presence of aPL in individuals without any clinical manifestations, i.e. aPL carriers, can be seen as a risk factor for first time thromboembolic events and various studies investigated the significance of aPL positivity and the occurrence of thrombotic events[6].

In more detail, LA has been shown to be a better predictor for thrombosis compared to any other aPL, as described by a systematic review in 2003 by Galli et al., including 753 patients and 234 controls, that showed that LA is a strong risk factors for both arterial and venous thrombosis[6]. On the other hand, De Groot et al. showed in their Leiden cohort that included 473 patients and 472 control subjects, that the presence of LA alone without the presence of anti-β2GPI (or antiprothrombin antibodies) was not significantly associated with a risk for a first deep vein thrombosis (DVT) (OR 1.3, 95 % CI 0.3–6.0). However, in patients who tested positive for LA and anti-β2GPI antibodies (or antiprothrombin) the OR of a first time deep venous thrombosis increased to 10.1 (95 % CI 1.3–79.8)[7].

Regarding triple positivity, Pengo et al. demonstrated the association of triple positive patients carries with an increased risk of clinical manifestations of APS, both thrombosis and adverse pregnancy outcome, compared to patients with positivity for only one aPL[8].

Otomo et al. expanded on this principle and developed the aPL-score (aPL-s), in order to determine whether aPL titres influence the risk of thrombosis, comparing high to medium/low titres of aCL and anti-β2GPI IgG and IgM, respectively. The group showed that high levels of IgG aCL, anti-β2GPI (and also antiphosphatidylserine and antiprothrombin antibodies) were closely related to the clinical manifestations of APS. In their study the aPL-score related with a history of thrombosis or pregnancy morbidity. Moreover, the aPL-s score was shown to be of predictive value for the recurrence and/or new onset of thrombotic events.

In conclusion, different aPL profiles are an important indicator for risk assessment of APS clinical manifestations and represent a fundamental tool for clinicians especially when managing aPL carriers.

3.1 Global APS Score (GAPSS)

Moving towards the concept of aPL as a risk factor, our group recently published a comprehensive series of studies developing and validating the global APS score (GAPSS) in different patients populations[9]. The GAPPS score combines independent risk factors for thrombosis and pregnancy loss, taking into account aPL profiles (criteria aPL and non-criteria aPL), as well as conventional cardiovascular risk factors and autoimmune antibody profiles. Among all the computed variables (extensive aPL testing, cardiovascular risk factors evaluation, autoimmune profile), multivariate logistic regression analysis showed that only arterial hypertension, hyperlipidaemia, LA, aCL IgG and/or IgM, anti-β2GPI IgG and/or IgM and aPS/PT IgG and/or IgM were independent risk factors for thrombosis and/or pregnancy morbidity.

In brief, all variables were computed as dichotomized, in order make GAPSS more widely adoptable. aPL positivity was assessed according to the updated APS classification criteria1. The GAPSS includes IgG/IgM aCL (five points), IgG/ IgM anti-β2GPI (four points), LA (four points), IgG/IgM anti-phosphatidylserine-prothrombin complex antibodies (three points), hyperlipedaemia (three points) and arterial hypertension (one point).

The GAPSS model was developed in patients with SLE and higher GAPS scores were observed in patients who experienced thrombosis and/or pregnancy loss compared with those without clinical events. Moreover, the GAPSS score was evaluated in a subsequent prospective study of 51 SLE patients[10] and in 62 consecutive patients with primary APS[10]. The GAPSS score was further The GAPSS model was further applied and validated by two independent groups that described APS manifestations (thrombosis or pregnancy morbidity) were experienced by patients with higher GAPSS values compared to patients without APS manifestations[10].

4. New therapeutic options beyond anticoagulation with Vitamin K antagonists

Long-term anticoagulation with Vitamin K antagonists (VKA) is currently the therapeutic option for thrombotic APS management. Table 1 summarizes the current strategies for management of patients with aPL. However, therapy with VKA rises many challenges for clinicians and patients, including adherence to treatment, dietary interactions and impacts on daily life. Besides, patients with APS might experience clinical manifestations not directly related to thrombotic pathogenesis, that may not respond to anticoagulation (e.g., from systemic symptoms such as fatigue to organ specific manifestations as cardiac valves involvement). Moreover, the monitoring of anticoagulation with VKA in LA positive patients might be challenging, as the responsiveness of the reagents used in the INR testing varies widely, leading to potential instability of anticoagulation. For the above reasons new therapeutic options for APS management are highly needed.

4.1 Hydroxychloroquine

Clinical trials and animal models supported the role of hydroxychloroquine (HCQ) as useful tool to reduce the risk of thrombosis[11]. The various antithrombotic mechanisms include inhibition of platelet aggregation and adhesion, cholesterol lowering mechanisms and blockade of aPL production[11].

In a recent trial, Rand et al. [12] showed that HCQ significantly reduced both the binding of aPL-b2GPI complexes to phospholipid surfaces and the binding of the individual proteins to bilayers. The same group demonstrated in a further study [12] that HCQ also caused modest, but statistically significant, reductions of clinical aPL titers as well as a reduction of the disruption by aPL of the annexin A5 anticoagulant shield.

Both retrospective and prospective studies demonstrated a beneficial effect of HCQ on primary thrombosis prevention in aPL-positive patients. More in detail, Kaiser et al.[13] enrolled in a large cohort of 1930 SLE patients, confirmed that HCQ use was protective for thrombosis. However, a recent randomized multicenter study (https://clinicaltrials.gov/ct2/show/NCT01784523)

investigating the prophylactic role of HCQ against thrombosis in patients with aPL has been early terminated due to low recruitment rate exacerbated by manufacturing shortage and price increase of HCQ, pointing out the challenges of designing prospective randomized trials in this conditions.

4.2 Rivaroxaban in Antiphospholipid Syndrome (RAPS)

The use of the novel anticoagulants would be expected to improve the quality of life of APS patients. These agents, in fact, have fewer drugs interactions and dietary restrictions compared with VKA and very predictable anticoagulants effects with fixed dosing regiments, making it unnecessary to routinely monitor anticoagulant intensity. However, the lack of INR monitoring could represent a downfall of novel anticoagulant therapy, introducing variability in patients adherence.

Rivaroxaban (a direct anti-X agent) has been approved by the European Medecines Agency for the prevention of stroke and systemic embolism in patients with atrial fibrillation and for venous thromboembolic events management. RAPS (Rivaroxaban in Antiphospholipid Syndrome) was a randomised, controlled, open-label, phase 2/3, non-inferiority trial, that included 116 APS patients who were taking VKA for previous venous thromboembolism, with a target international normalised ratio of 2.5[14]. Patients were randomized to receive warfarin or 20 mg oral rivaroxaban daily. Treatment effect was measured as the ratio of rivaroxaban to warfarin for thrombin generation. Endogenous thrombin potential for rivaroxaban did not reach the non-inferiority threshold, but as there was no increase in thrombotic risk compared with standard-intensity warfarin. No thrombosis or major bleeding were seen. Serious adverse events occurred in four patients. In brief, the first was an intracranial haemorrhage that pre-dated the trial, the second was an episode of abdominal pain, vomiting, arthralgia, and myalgia. Two were judged to be unrelated to the trial drug: the first was a suspected deep vein thrombosis at day 176, the second was intestinal perforation.

Rivaroxaban could be an effective alternative in patients with APS and previous venous thromboembolism. Its use in APS patients with arterial events and/or high risk aPL profile needs further investigation. To date, at least other three trials (RAPS-Canada, TRAPS (https://clinicaltrials.gov/ct2/show/NCT02157272) and ASTRO-APS (https://clinicaltrials.gov/ct2/show/NCT02295475) are currently further investigating the use of direct new oral anticoagulants in patients with APS and results are highly waited.

4.3 IVIG

Evidences support the efficacy of IVIG in addition to conventional therapy, in primary and secondary APS patients, in preventing the occurrence of further thromboembolic events[15]. Despite the promising observations especially in patients with the so-called APS *plus (*APS with ANA positivity and sign/symptoms of a connective tissue disease not fulfilling the classification criteria) further data are need to establish indications and optimal doses of IVIG in thrombotic APS.

4.4 B-Cells depletion therapy

While immunosuppressive drugs such as i.v. cyclophosphamide might be helpful in patients with active systemic autoimmune disease mainly SLE and systemic vasculitis, their use in APS is still controversial and limited to very selected case of catastrophic APS (CAPS) or in severe cases refractory to standard therapy. Some case reports about the use of rituximab – an anti-CD20 monoclonal – in the treatment of APS have been published[16].

B cells are likely to play a central role in the generation of the aPL-induced clinical manifestations of the disease, so could constitute a logical therapeutic target in APS. Anecdotally, its use has also been associated with a down-regulation of aPL titers[17]. A pilot open-label phase II trial of RTX for noncriteria manifestations of APS (such as thrombocytopenia, skin ulcers, nephropathy and cognitive dysfunction) concluded that RTX may represent a well-tolerated option in the therapeutic arsenal for APS. However, it has been reported to be effective in controlling some but not all non-criteria manifestations of APS. Overall, although more data are necessary to support the use of these

drugs in the setting of severe APS, current experience seems quite promising, especially in patients with severe thrombocytopaenia. Table 2 summaries our experience with cyclophosphamide and/or rituximab in very life-threating cases refractory to standard therapies. Of note, two patients have been treated with an Intensified B-Cell Depletion Therapy (IBCDT), an approach we employed as a rescue therapy in refractory lupus nephritis or systemic vasculitis. IBCDT consisting of "four (weekly) plus two (monthly) doses" of rituximab (375 mg/sm), associated with two i.v administrations of 10 mg/kg cyclophosphamide and three pulses of 15 mg/kg methylprednisolone, without further immunosuppressive maintenance therapy. Despite the promising results, this approach has been limited to very selected cases and further data are needed to investigate dosage and indications.

4.5 Eculizumab

Eculizumab is a recombinant full-humanized IgG2/IgG4 monoclonal antibody that blocks the formation of the terminal complex sC5b-9 and C5a by binding to the C5 complement component and consequently blocking the activation pathway. Growing evidences from *in vitro* and *in vivo* studies are suggesting a promising role for eculizumab for APS. In fact, the complement can be activated by the binding of C3 fragment to the Fc receptor of aPL antibodies. The activation of complement pathway and consequently production of inflammatory molecules like C5a by aPL can directly activate platelets and monocytes, inducing the coagulation cascade and leading to thrombosis. Recently, Durcan and al[18] observed in a cohort of 2399 patients that the presence of aCL and hypocomplementemia (both low C3 and C4) strongly associates with deep vein thrombosis. The presence of LAC and low C4 were also associated with stroke. Therefore, eculizumab might represent a valuable therapeutic alternative in APS patients, especially in patients with concomitant hypocomplementemia.

Furthermore, case reports describe the successful use of eculizumab in severe cases of APS, such as the catastrophic variant of the syndrome and cases of APS and thrombotic microangiopathy[19]

5.Conclusion

APS remains a substantial diagnostic challenge for physicians, mainly due to the expanding range of reported clinical manifestations associated with the presence of aPL as well as to the expanding limitations of current laboratory testing. Although it is the physician taking care of the patient who ultimately makes the diagnosis, laboratory-testing still plays a key role in many phases of the management. While it is widely accepted that aPL play a crucial pathogenic role in inducing clinical manifestations, limitations in detailed knowledge by both clinical and laboratory perspectives regarding the "complete" range of available aPL tests, as well as ongoing problems with assay reproducibility and standardization exist.

To date, aPL profiling represents the most accurate risk stratification tool for thrombosis. The so called "triple positivity" was found to be associated with thrombosis in up to 87% of cases of APS while in the other profiles the association was around 50%[20]. With regard to risk stratification, some scoring systems have been proposed to help physicians to identify the individual risk of thrombosis/pregnancy morbidity in patients positive for aPL; among others, the GAPSS, which brings together the aPL profile (including both criteria and non-criteria aPL) and traditional cardiovascular risk factors, seems a promising tool to identify patients at higher risk of new events. Referring to treatments, while current therapeutic options remain confined to long-term anticoagulation with VKA, the future holds much promise with the identification of novel potential targets, many of which are currently under investigation. The challenge will be to design prospective randomized controlled clinical trials to provide the evidence necessary to support integration of these therapies into clinical practice. Ideally, the task for the future will be to tailor the APS management, taking into account aPL profile and clinical manifestations.

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Authorship Contributions

SS and MR searched the literature, assisted with the organization of the manuscript, interpreted and collected data, and wrote and edited the manuscript. MB, DR and SS interpreted and collected data, helped to design the figures and panel, and wrote and edited the manuscript.

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