Lupus anticoagulant: a never-ending story. New insights from anti prothrombin antibodies

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ABSTRACT

Until recently, the lupus anticoagulant (LA) phenomenon was largely attributed to antibodies directed against β2-glycoprotein I (β2GPI). However, this explanation does not account for LA activity in patients with isolated LA, in whom anti-β2GPI antibodies are absent. Increasing evidence indicates that anti-prothrombin (aPT) antibodies play a key role in these cases. aPT antibodies detected with the anti-phosphatidyl-serine/prothrombin (aPS/PT) test appear to explain the coagulation inhibition observed in most patients with isolated LA. They also represent a major determinant of LA activity in triple-positive patients (LA, aCL, and aβ2GPI). Because false-positive LA results frequently occur in patients receiving oral anticoagulants, detection of aPT antibodies may represent a useful surrogate marker of LA. *In vitro*, some aPT antibodies can induce activated protein C resistance, contributing to the hypercoagulable state promoted by aβ2GPI antibodies. However, aPT antibodies are also involved in LA/hypoprothrombinemia syndrome, which is a bleeding disorder. In this case, aPT antibodies contribute to causing severe hypoprothrombinemia by promoting rapid prothrombin clearance. These observations suggest three biological patterns of LA: i) procoagulant LA in triple-positive patients at high thrombotic risk, typically associated with high-titer IgG aPT antibodies detected by aPS/PT test. ii) Mildly procoagulant LA in patients with isolated LA, generally associated with moderate-to-high-titer IgM aPT antibodies detected by aPS/PT test. iii) Anticoagulant LA, characterized by very high titers of aPT antibodies detected by aPS/PT test associated with hypoprothrombinemia and bleeding. Recognition of these mechanisms is refining the interpretation of LA and providing new insights into the pathophysiology of antiphospholipid syndrome.

Key words: lupus coagulation inhibitor, antibodies, prothrombin, thrombosis, bleeding.

Introduction

The lupus anticoagulant (LA) is considered the strongest laboratory risk factor for thrombosis in patients with antiphospholipid syndrome (aPS). Paradoxically, although LA prolongs the phospholipid-dependent coagulation assays *in vitro*, it is associated with an increased risk of thromboembolic events *in vivo*.[1]

Because coagulation times shorten after incubation of patient plasma with excess phospholipids (PLs), early research focused on antibodies directed against PLs, particularly cardiolipin.[2]

In 1990, three independent groups demonstrated that anti-cardiolipin antibodies (aCL) are not directed against cardiolipin itself but against a plasma protein that binds phospholipids: β2-glycoprotein I (β2GPI).[3-5] Subsequent studies showed that anti-2GPI antibodies (aβ2GPI) directed towards the Domain 1 are strongly associated with thrombosis.[6]

At that stage, three laboratory tests were available to detect antiphospholipid antibodies (aPL): LA; aCL, and aβ2GPI.

It soon became clear that simultaneous positivity in all three assays (*triple positivity*) is strongly associated with thromboembolic events.[7]

In triple positive patients LA activity was formerly explained by the presence of aβ2GPI but anti-prothrombin antibodies could also contribute to LA activity.[8] In the early 2000's, assays detecting antibodies against the phosphatidylserine/prothrombin complex (aPS/PT) in the presence of calcium ions were developed and demonstrated the presence of such antibodies in patients with LA.[9] Concurrently, efforts were made to develop laboratory tests capable of distinguishing LA associated with aβ2GPI antibodies from that associated with anti-prothrombin antibodies (aPT).[10,11]

Antibody patterns in triple-positive antiphospholipid syndrome

Two main antibodies characterize triple positive patients: those directed against β2GPI and those directed against PT.[12] Both

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antibodies contribute to the acquired thrombophilia observed in these patients. aβ2GPI promote thrombosis primarily through platelet activation.[13-15] In contrast, some aPT antibodies can induce activated protein C resistance, thereby contributing to the hypercoagulable state.[16]

When present alone, aPT antibodies may induce a mild procoagulant state, which may explain the lower incidence of thromboembolic events observed in carriers of isolated LA compared with patients with triple positivity.[17,18]

More recently, we demonstrated that aPT antibodies detected with aPS/PT test are frequently present in triple positive patients (*tetra-positivity*) and represent a major contributor to LA activity in patient plasma.[19] In addition, a good correlation was observed between LA potency [measured by Russell's Viper Venom time (RVVT) mixing/diluted RVVT control ratio] and aPS/PT antibody titers.[20]

Diagnostic challenges in lupus anticoagulant testing

LA testing remains a technically complex and poorly standardized procedure. A survey evaluating 302 positive plasma samples sent to our reference laboratory revealed that 71 (23%) were false positive results.[21] Misdiagnosis was significantly associated with: first-time tested in the lab; LA reported as weak or borderline and testing performed in patients receiving oral anticoagulant therapy.

Other recognized causes of diagnostic errors are reported in Table 1.[22-30]

Overcoming anticoagulant interference in lupus anticoagulant testing

To overcome the interference from oral anticoagulants in LA detection, two main strategies have been explored.

One approach involves snake venom-based coagulation assays. Venoms from Coastal Taipan (*Oxyuranus scutellatus*) and the Indian saw-scaled viper (*Echis carinatus carinatus*) directly activate prothrombin, bypass the effects of vitamin K antagonists and direct factor Xa inhibitors.[31,32] Because the Taipan Venom time is PL-dependent, it is used as screening test, while the PL-independent Ecarin time is used as a confirmatory test.

Another strategy involves *in vitro* absorption of direct oral anticoagulants (DOACs) using activated charcoal compounds, which has promising results for LA-detection in patients receiving DOAC therapy.[33]

Anti-prothrombin antibodies as a surrogate marker of lupus anticoagulant

A recent meta-analysis reported that the pooled prevalence of IgG or IgM aPT antibodies detected by the aPS/PT test in LA-positive patients was 84.6%.[34] However, the methodological quality of the included studies was suboptimal. False-positive LA or aPS/PT level insufficient to prolong coagulation assays may have weakened the observed association between LA and aPS/PT antibodies. A better correlation will be found when aPS/PT antibody profiling will be made on automated solid-phase chemiluminescence platforms, which provide more accurate and standardized detection than conventional ELISA, reducing operator-dependent variability and improving workflow efficiency.

Nevertheless, these findings suggest that aPT antibodies detected by the aPS/PT test may serve as a useful adjunct in the diagnostic work-up of aPS, particularly when LA results are borderline or potentially unreliable.

Testing for aPS/PT may not be necessary when LA is clearly positive in two assays and confounding factors are absent. However, it may be valuable when LA results are borderline, discordant, or potentially affected by anticoagulant therapy (Figure 1).

Role of anti-prothrombin in isolated lupus anticoagulant

The presence of aPT antibodies may explain LA activity not only in triple positive patients but also in those with isolated LA, defined as the absence of aβ2GPI antibodies.[12]

We investigated this hypothesis in a patient with psoriasis who presented with markedly prolonged Silica Clotting Time and dRVVT. Mixing studies confirmed the presence of an inhibitor, and confirmatory studies corrected the prolongation. Both aCL and aβ2GPI antibodies were negative. Fractionation of patient plasma demonstrated both LA activity and IgM aPS/PT antibodies. Similar findings were subsequently observed in 25 patients with isolated LA.[12] These results indicate that aPT antibodies are responsible for LA activity in many patients lacking aβ2GPI antibodies.

Clinical situations in which anti-phosphatidylserine/prothrombin antibodies testing may be useful

Several clinical scenarios illustrate the potential clinical utility of detecting aPT using aPS/PT test.

In a 40-year-old woman with recurrent transient ischemic attacks of unknown origin, screening revealed double positivity for

Table 1. Diagnostic challenges on the laboratory detection of lupus anticoagulant.

LA false-positive	When reported as weak or borderline.[21] Presence of anticoagulants (VKA, direct oral anticoagulants, LMWH) in testing plasma.[22,23] Presence of antibodies to specific coagulation factors.[24] Increased levels of C-reactive protein (through its affinity for phospholipids).[25]
LA false-negative	Type of reagents or excess residual platelets in tested plasma.[26] Increased FVIII level.[27] Presence of Lupus Cofactor.[28]
LA of uncertain interpretation	Only one test positive with potential misdiagnosis.[29,30]

LA, lupus anticoagulant; VKA, Vitamin K antagonists, LMWH, low molecular weight heparins.

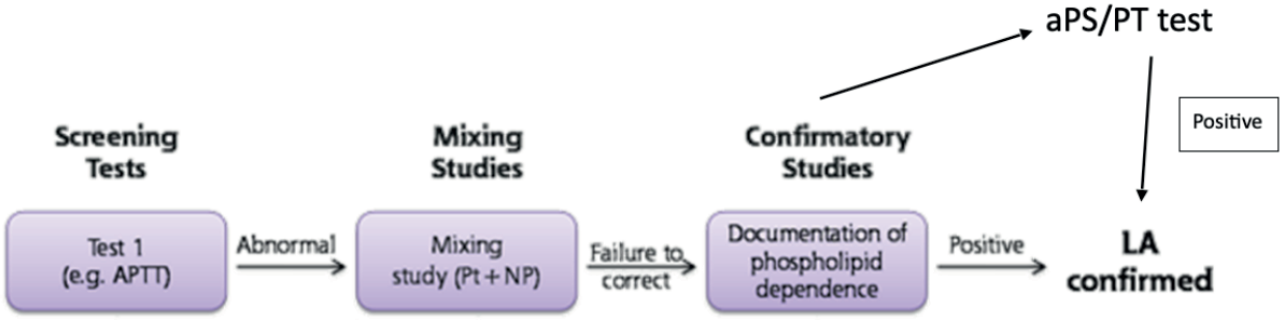

Figure 1. Schematic representation of steps allowing lupus anticoagulant identification. An anti-phosphatidyl-serine/prothrombin test could help identify the inhibitor.

IgG aCL and IgG aβ2GPI with negative LA. Testing for IgG and IgM aPS/PT were negative confirming the absence of LA and supporting individualized therapeutic decision-making.[35] In such cases, testing for IgG anti-β2GPI Domain I antibodies (aβ2GPI/Dm1) may further help determining whether single or dual antiplatelet therapy is appropriate.[36]

Another situation involves young patients presenting with unprovoked venous thromboembolism.

Because anticoagulant therapy is often initiated immediately, LA testing becomes unreliable. In this setting, measurement of aCL, anti-β2GPI and aPS/PT antibodies, which are not affected by anticoagulant therapy, may help guiding treatment decisions. When all the tests are positive, long-term treatment with vitamin K antagonists should be considered.[37]

Finally, aPT antibodies play a central role in the diagnosis of LA /hypoprothrombinemia syndrome (LAHS). In this condition, extremely high antibody titers lead to rapid prothrombin clearance a clinical condition where the main clinical picture is bleeding and not thrombosis. Usually the titer of aPS/PT in these cases is very high, resulting in hypoprothrombinemia and bleeding manifestations.[28,38]

Toward a new interpretation of lupus anticoagulant

Taken together, these findings suggest that the traditional concepts of lupus anticoagulant may require consideration. The umbrella term *lupus coagulation inhibitor* used in Medical Subjects Headings may manifest in different clinical phenotypes depending on antibody profile and characteristics (Figure 2). When associ-

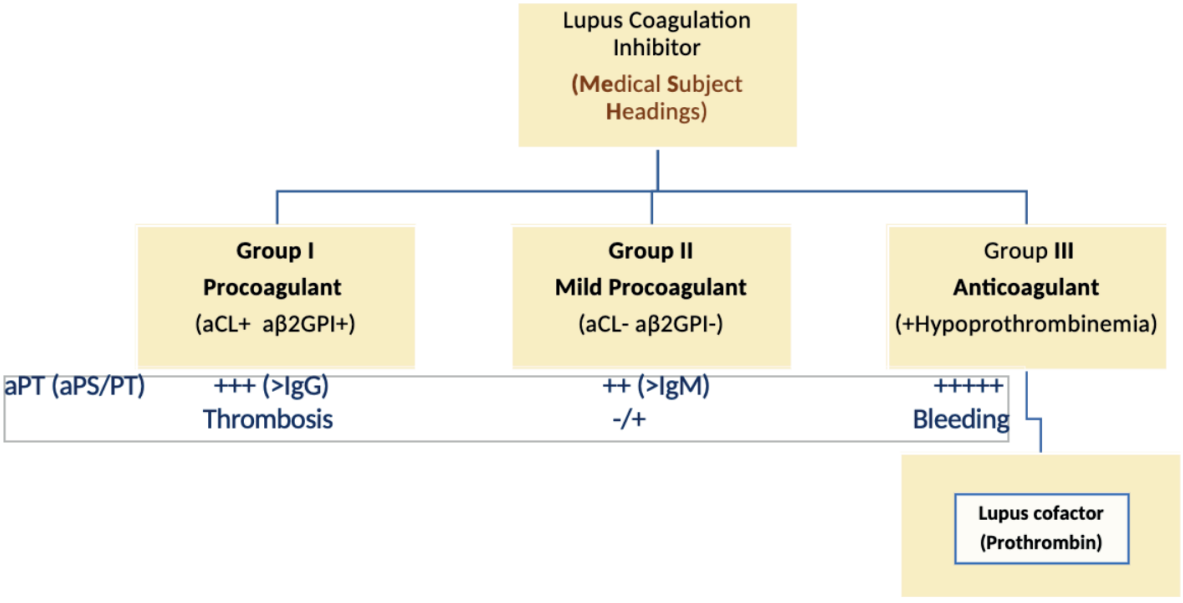

Figure 2. Different types of lupus coagulation inhibitor. Group I (procoagulant) is associated with anticardiolipin antibodies (aCL) and aβ2GPI and frequently with IgG antiphosphatidylserine/prothrombin complex (aPS/PT; tetra positivity) and thrombosis. Group II (mild procoagulant) is not associated with aCL and aβ2GPI and aPS/PT are often of IgM isotype, and thrombosis is less frequent than in Group I. Group III could be associated with aCL and aβ2GPI but the main characteristic is very high level of aPS/PT, determining a reduction of prothrombin to levels inducing bleeding. The lupus cofactor phenomenon is frequent (prolongation of coagulation tests in mixing studies).

ated with aCL, anti-β2GPI and aPT antibodies, the inhibitor promotes a strong prothrombotic state typical of high-risk APS. When aPT are present alone, the resulting thrombophilia appears milder. Conversely, very high titers of aPT antibodies may induce hypoprothrombinemia and bleeding, as observed in LAHS. In this case, it is frequent the phenomenon of Lupus Cofactor (increase of coagulation time in mixing studies) due to supply of antigen (prothrombin) to anti-prothrombin antibodies.[39]

Emerging biochemical insights

In recent years, structural and biochemical studies have further refined our understanding of aPT antibodies in the context of LA. Two subpopulations have been identified in high-risk patients.[40-42] Type I antibodies appear weak LA inducers and have no APC-resistance, whereas Type II antibodies show strong LA and cause APC-resistance. These findings are providing new insights into the mechanisms linking aPT antibodies with coagulation dysregulation and thrombosis and may open new avenues for refined detection and a deeper understanding of the pathogenesis of APS.

Conclusions

Accumulating evidence indicates that aPT antibodies are key determinants of lupus anticoagulant activity. They contribute to LA expression in both triple-positive APS and isolated LA and may also explain the paradoxical bleeding observed in lupus anticoagulant hypoprothrombinemia syndrome. Incorporating aPT testing into selected clinical and laboratory scenarios may improve the interpretation of LA results and refine the diagnostic work-up of APS. Further research is needed to clarify the biological diversity of aPT antibodies and their role in the pathogenesis of thrombosis and bleeding in APS.

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