



VIEWPOINT

Escaping the catch 22 of lupus
anticoagulant testingPernille Just Vinholt ¹, Søren Andreas Just ²

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ABSTRACT

High-risk patients with antiphospholipid syndrome (APS) experience increased risk of thrombosis when treated with direct oral anticoagulant (DOAC) therapy compared with warfarin. It is essential to establish the APS diagnosis to choose therapy and determine treatment duration. It requires testing for antiphospholipid antibodies, including lupus anticoagulant (LAC). In this viewpoint, we discuss the options for timing of LAC testing, which includes testing before starting anticoagulant treatment (DOAC or warfarin), after switching to heparin or after withdrawal of anticoagulant treatment. DOACs interfere with LAC testing and recommendations emerge stating not to conduct on-therapy LAC testing. All approaches are to some extent currently practised, but have limitations and the area is therefore seemingly a catch 22. We put forward that the anticoagulant effect of DOAC can be eliminated in the laboratory and therefore patients can be tested on-therapy. While it may not eliminate all cases of interference, it could aid the interpretation in these situations and this approach is attractive from the patient and clinician's perspective. Nevertheless, to prevent misdiagnosis the diagnostic workup for APS requires collaboration between the clinician and the laboratory. We advocate for standardisation in laboratory and clinical practice when diagnosing APS.

DIAGNOSE OF ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS) is a clinical condition in patients with episodes of arterial thrombosis, venous thrombosis or pregnancy complications and presence of one or more of three types of antiphospholipid antibodies (aPL): lupus anticoagulant (LAC), anti- β 2-glycoprotein 1 (β 2-GPI) or anticardiolipin antibodies (aCL) demonstrated 12 weeks apart.^{1,2} LAC is an in vitro phenomenon observed when antibodies interfere with phospholipid-dependent laboratory analyses resulting in elongation of clotting times.¹ To rule out LAC, negative results with two methods are required.¹ Most laboratories use dilute Russel's Viper Venom Time (dRVVT) as first-line test and activated partial thromboplastin time (APTT) based analysis as second-line test.² High titres of aPL and triple positivity for LAC, aCL and anti- β 2GPI are associated with high thrombotic risk.³

THROMBOTIC RISK AND TREATMENT IN PATIENTS WITH APS

The vitamin K antagonist, warfarin, has been the preferred treatment of thrombosis in patients with APS. However, the use of direct oral anticoagulant (DOAC) has been highly profiled and surpassed the use of warfarin for venous thromboembolism treatment.⁴ DOACs comprise dabigatran, a thrombin inhibitor; apixaban, edoxaban, rivaroxaban, which are factor Xa inhibitors. As DOACs do not need monitoring and are associated with lower risk of fatal bleeding than warfarin, their use is appealing. However, Pengo *et al* recently showed that DOAC treatment is associated with higher risk of arterial thrombosis than warfarin in patients with previous thrombosis and triple aPL positivity (LAC, aCL and β 2-GPI).⁵ The study was stopped early do to this observed imbalance.⁵ It is still uncertain whether DOACs are safe for some patients with APS, for example, for those who test positive for one or two aPLs or have aPL in low titre.^{6,7} Nevertheless, novel guidelines recommend that DOACs are not used in patients with APS and triple aPL positivity.⁸

HOW TO TIME TESTING FOR ANTIPHOSPHOLIPID ANTIBODIES?

Patients with thromboembolism can be candidates for aPL testing.⁹ Theoretically, sampling could be conducted before commencing treatment. While it is to some extent practised to request thrombophilia testing shortly after the thrombotic event,² there is a risk of false positive results due to ongoing coagulation activation and interference from drugs.^{1,10,11} Even when LAC is evaluated before commencing anticoagulation, the LAC test shall be repeated after 12 weeks time to establish a clinical diagnosis of APS.¹ Instead testing can be timed to a period after withdrawal of anticoagulant treatment. However, interruption of anticoagulation will expose the patients to increased thrombotic risk. These limitations also apply for warfarin



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treatment. It is possible to switch to heparin ahead of dRVVT analysis, but the praxis is laborious, difficult to administer and thus not appropriate as a general recommendation.² Further, APTT-based LAC analyses cannot be performed, which makes it difficult to rule out LAC if dRVVT is negative. The last option is to test patients on anticoagulant treatment. However, it has emerged, that a high rate of false results in LAC-testing is observed with all DOACs and thrombin inhibitors, especially seen in samples with rivaroxaban.^{12–17} Most in vitro studies found that LAC results become false-positive. False-negative LAC results in samples with apixaban has been proposed in a study based on retrospective review of laboratory data.¹⁷ Interference was observed even for samples spiked with DOAC in concentrations corresponding to through levels and below the limit of detection of commercially available tests for DOAC concentration measurements; it applied for both dRVVT and APTT-based methods.^{13,18} In vivo studies support these findings.^{12,14,19} Therefore, the opinion emerges, that testing for LA should not be done while patients receive DOAC.^{2,20,21}

So, can we escape this apparent catch 22? We need the test but cannot get reliable results. One simple way to handle these obstacles would be to remove the anticoagulant and/or the anticoagulant effect from the sample prior to analysis. The DOAC-STOP (Haematex Research, Hornsby, Australia) is an insoluble commercial adsorbent material that eliminates the anticoagulant in vitro.¹⁵ DOAC-STOP can be added to samples before testing and it does not affect dRVVT in patients who do not receive DOACs.²¹ When using DOAC-STOP, the results from patients in DOACs can be interpreted.^{15,19,22–24} A simple charcoal product (DOAC-Remove, 5-Diagnostics, Basel, Switzerland) may offer an alternative solution to eliminate anticoagulation effects before LAC testing.^{16,25} Another strategy would be to add specific reversal agents pre-analytically. Idarucizumab is a humanised monoclonal antibody fragment, which was equally effective as DOAC-STOP for reversal of dabigatran.²⁶ Andexanet Alfa is a modified physiologically inactive human factor Xa decoy protein that binds factor Xa inhibitors with high affinity. However, the reversal of rivaroxaban with Andexanet Alfa did not eliminate the anticoagulant effect enough to prevent interference in LAC.²⁷ Overall, elimination of DOACs in samples seems to be an option and could be combined with testing at estimated time for through DOAC levels. Finally, the sensitivity for DOACs differ among reagents for LAC and Taipan snake venom time/ecarin time might to some extent be used for patients on warfarin or rivaroxaban.²⁸ Thus, there is an urgent need for standardisation, as quality assessments have reported false LAC results rate of 10%–50% with current praxis.²

CLINICIAN'S PERSPECTIVE

Current guideline states that testing should be limited to patients who have a significant probability of having the APS.¹ However, it is increasingly clear that aPL testing could be relevant for all patients with thromboembolism, because the results have an impact on

treatment choice and duration. In clinical practice, the indication for testing is often the choice of the clinicians. They may not all be aware of the limitations of LAC testing.² Thus, the information regarding ongoing DOAC treatment might be lacking when the blood sample is received in the laboratory and overlocked when the results are interpreted. Therefore, it may be necessary to always have a reversal procedure included in the setup. While false-negative LAC results have been reported, reversal is relevant at least for initially positive results. Even if interference from DOACs is not eliminated in all situations, the frequency of positive LAC results would be minimised with the suggested procedure. These few LAC positive samples could result in the shift of therapy to warfarin or bridging with heparin before repeat LAC testing. In any case, a close contact between clinicians and the laboratory needs to be established to collaborate on LAC testing.²¹

CONCLUSION

In conclusion, patients testing triple positive for aCL, anti- β 2GPI and LAC are in high risk of thrombosis and should not receive DOAC but extended anticoagulation with warfarin after experiencing thrombosis. There is a clinical need for standardisation regarding how to time and manage LAC testing during ongoing anticoagulant treatment. We propose that the anticoagulant effect is sought eliminated preanalytically, which could reduce the number of false-positive LAC results combined with procedures for retesting initially positive samples is safe for the patient and feasible for the clinician. It highlights the importance of a close collaboration between the clinicians and their laboratory preventing patients from being caught in the catch 22 of lupus anticoagulant testing.

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