THE PATHCARE NEWS

Lupus anticoagulant in Antiphospholipid Syndrome

Definition:

Antiphospholipid syndrome (APS) is defined by **venous or arterial thrombosis and/or pregnancy loss or morbidity** in the **presence of persistent antiphospholipid antibodies** (aPLs) namely anti β-2 glycoprotein I (anti β-2 GPI), anticardiolipin (aCL) and lupus anticoagulant (LA). These acquired autoantibodies are directed against proteins that are bound to anionic phospholipids. The main protein found to be targeted in APS is the β-2 glycoprotein I (β-2 GPI). However, other proteins like prothrombin, annexin 5A or phospholipids like phosphatidyl-ethanolamine are also targeted by these autoantibodies[1]. The mechanism by which these antibodies cause thrombosis is not completely understood. Clinically there is also a wide variety in clinical manifestations of APS. The only defining feature is the persistent presence of one or more aPLs. Thus the laboratory investigations become critical in the diagnosis.

Diagnostic criteria:

Due to lack of specific clinical associations of APS, diagnostic criteria are used. This requires the history of **one clinical event** and the presence of **one laboratory positive antiphospholipid antibody**[1, 2].

The accurate laboratory testing of APS carries significant consequences for the individuals with clinical manifestations that can be attributed to the disease. In the setting of venous thrombosis (VTE), this is lifelong anticoagulant use with its associated accumulative risk of bleeding. In arterial thrombosis treatment with antiplatelet agent or an anticoagulant such as vitamin K antagonist like warfarin would be considered [1].

Indications for Laboratory testing:

There should be a **significant probability of APS before testing** so as to minimize finding incidental LA and false positives.

Grading of the appropriateness of test for antiphospholipid antibodies:

Low:	Venous or arterial thromboembolism in elderly patients.
Moderate:	Accidentally found prolonged PTT in asymptomatic patients.
HIGH:	Unprovoked VTE and arterial thrombosis in young patients (< 50 years), thrombosis at unusual sites, any thrombosis or pregnancy loss or pregnancy morbidity in patients with autoimmune diseases (SLE, rheumatoid arthritis, autoimmune haemolytic anaemia or autoimmune thrombocytopenia) and late pregnancy losses [3].

Laboratory diagnosis:

The laboratory tests used are:

1. Solid based tests, (ELISA method)
   a. Anticardiolipin (aCL) and
   b. Anti β-2 glycoprotein I (Anti β-2 GPI) [Test code: K1171]
2. Coagulation based test
   a. Lupus anticoagulant (LA) [Test code: X1137]

Both these tests need to be requested for analysis.

A **positive** result must be present on **two or more occasions** at least **12 weeks apart.** Repeat testing must be done to exclude transient antibodies which are present due to other reasons such as drugs or infections.

In depth focus on Lupus Anticoagulant testing in the laboratory

Lupus anticoagulant (LA) is a heterogeneous antibody that causes prolongation of clotting times in the plasma of patients in vitro. This LA phenomenon is due to inhibition of proteins that are bound to anionic phospholipids. The specific antibodies responsible are anti β-2 GPI and anti-prothrombin (aPT). The word anticoagulant is a misnomer as LA is mostly seen in setting of thrombosis.

The LA positivity due to anti β-2 positivity alone correlates more strongly with thrombosis than positivity to ELISA based tests alone. Also individuals with LA positivity have higher titres of anti β-2 GPI and aCL antibodies than individuals with anti β-2 GPI and aCL antibodies alone. Thus it is thought that the strong association of thrombosis to LA is due to higher titres[2]. The positivity of more than one aPL (particularly triple positivity; LA, aCL and anti-β2 GPI) carries higher risk of thrombosis than positivity of only one aPL. Due to the heterogenic nature of lupus anticoagulants, reagent- and analyser variability as well as the lack of gold standard in the detection of LA, no one test currently can detect all clinically significant LAs. Guidelines have been established for Laboratory Detection of Lupus anticoagulant (BCSH, CLSI and ISTH). Two assays are recommended which are based on different analytical principles (intrinsic, common and extrinsic pathway). This is done to improve specificity and also to ensure that weak LAs are detected. The combined use of these two tests increase the detection rates to acceptable levels. However, the risk of false positive results is inherent due to poor specificity of the tests. The challenge with LA testing is that we are detecting an antibody by inference after excluding all other causes of a prolonged clotting time in a patient. Thus it becomes prudent to do preliminary tests (PTT, TT, INR) to exclude these possibilities. These tests are not always requested by clinicians, which then decrease the specificity.

LA assays:

Pairing of dilute Russel viper venom time (dRVVT) and LA responsive activated partial thromboplastin time (aPTT) or modified aPTT assays are recommended. dRVVT has been found to be more sensitive to β-2GPI dependent antibodies and has been strongly associated with thrombosis [3,4].

PathCare use dRVVT and Silica clotting time (SCT) assays. The principle of both of these test are:

1. Prolongation of a phospholipid dependent clotting test (screening test).
2. Demonstration of an inhibitor or acquired factor deficiency (mixing test).
3. Demonstration of the phospholipid dependence of the inhibitor (confirmatory test).

Reporting of the LA tests:

The tests are reported as normalized ratios according to recommended guidelines[3]. This is to eliminate analyser and operator performance variations. On the report each test has a comment regarding the interpretation whether LA is present or not detected.

Note: For the **diagnosis of LA** detection only **one test needs to be positive** on **two or more occasions.** Thus a **repeat test in no less than 12 weeks is mandatory[5].**

Testing in anticoagulated patients:

Testing for LA in patients receiving vitamin K antagonists like warfarin and therapeutic doses of unfractionated heparin should be postponed until anticoagulation has been discontinued for a suitable period (usually 1 - 2 weeks). This is because of the difficulty in interpretation due to prolonged basal clotting times. Similarly dabigatran and rivaroxaban affect tests for LA not only in screening and mixing, but also in confirmatory studies. Therefore LA testing should not be performed when patients are taking these drugs, particularly if blood is collected at peak, in order to avoid false-positive results[6]. Bridging with low molecular weight heparin (LMWH) can be employed in high risk patients[7].

Please contact your local PathCare haematopathologist, should you require additional information.

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References

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