The SSC algorithm for lupus testing

Gentian Denas MD

Cardiology Clinic

Padua University Hospital

CARDIOLOGY CLINIC PADUA

Journal of Thrombosis and Haemostasis, 7: 1737–1740 DOI: 10.1111/j.1538-7836.2009.03555.x

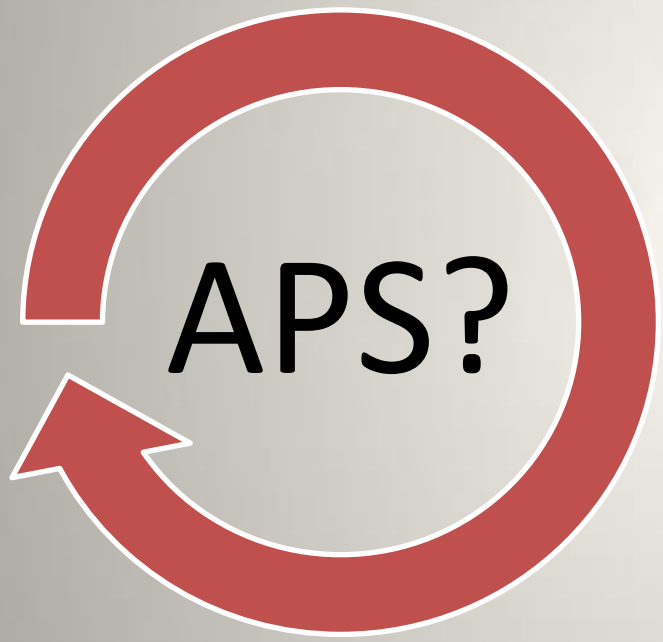
OFFICIAL COMMUNICATION OF THE SSC

Update of the guidelines for lupus anticoagulant detection

V. PENGO,* A. TRIPODI,† G. REBER,‡ J. H. RAND,§ T. L. ORTEL,¶ M. GALLI** and P. G. DE GROOT††

**Clinical Cardiology, Thrombosis Center, University Hospital, Padova; †Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, University and IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy; ‡Haemostasis Unit, Division of Angiology and Haemostasis, University Hospital, Geneva, Switzerland; §Hematology and Advanced Coagulation Laboratory, Montefiore Medical Center, Bronx, NY; ¶Division of Hematology, Duke University Medical Center, Durham, NC, USA; **Department of Hematology, Ospedali Riuniti, Bergamo, Italy; and ††Department of Clinical Chemistry and Haematology, University Medical Centre, Utrecht, the Netherlands*

Antiphospholipid syndrome flow



- **Clinical evidence**
- **Lab tests (LA** and others**)**
- Patient risk profile

Patient selection

- Testing for **LA** should be limited to patients who have a **significant probability** of having the antiphospholipid syndrome

OR

- Have unexplained prolonged aPTT during routine laboratory testing

Appropriateness of search for LA

- ***Low:*** venous or arterial thromboembolism in elderly patients
- ***Moderate:*** accidentally found prolonged aPTT in asymptomatic subjects, recurrent spontaneous early pregnancy loss, provoked VTE in young patients
- ***High:*** unprovoked VTE and (unexplained) arterial thrombosis in young patients, thrombosis at unusual sites, late pregnancy loss, any thrombosis or pregnancy morbidity in patients with autoimmune diseases

SSC flow for LA detection

- **Screening:** prolongation of a PL-dependent clotting assay (**dRVVT APTT**)

If the results are above local cut-off value and heparin can be excluded (thrombin clotting time)

If the results are above the local cut-off value

- **Mixing:** a 1:1 proportion of patient's plasma and a normal pooled plasma

- **Confirmation:** that the inhibitory activity is phospholipid-dependent

If % correction (ICA) is above local cut-off value

LA confirmed

Blood collection

- **Blood collection** before the start of any **anticoagulant drug** or a sufficient period after its discontinuation
- Fresh venous blood in 0.109 M sodium citrate 9:1
- **Double centrifugation** to obtain platelet free plasma
- Quickly frozen plasma is required if LA detection is postponed
- Frozen plasma must be thawed at 37°C

Screening assays

- **Two** tests that have different assay principles should be used in order to account for antibody heterogeneity
- **dRVVT** should be the first choice
- LA-sensitive **aPTT** (low PL and **silica as activator**) should be a second choice
- Further testing feasible if one of the two tests gives a positive result

Other tests are **not recommended** due to the low *sensitivity* (Ellagic acid), *variability* in thromboplastin reagents (dPT), lack of *standardised* commercial assays (Ecarin and Textarin), and poor *reproducibility* (Kaolin)

Mixing assay

- A mix of 1:1 proportion of the patient's plasma and a normal pooled plasma without preincubation
- The clotting assay used in the screening test is reassessed with this method

False positivity due to contamination (or heparin therapy) can be ruled out by performing the thrombin time (TT) on the patient's plasma

Confirmation assay

- **Confirmatory test(s)** must be performed by increasing the concentration of PL of the screening test(s) using either bilayer or hexagonal (II) phase PL
- Perform testing on plasmas from healthy donors at low (screen) and high (confirm) PL concentration
- Take the **cut-off** as the value corresponding to the mean of the individual % corrections calculated as [(screen-confirm)/screen]x100
- **Results** are confirmatory of LA if the % correction is above the local cut-off value

Cut-off values and results

- The **cut-off values** (screening and mixing tests) are taken as values above the **99th percentile** of the distribution
- The **results** (of all tests) should be expressed as the **ratio of the test result/normal pooled plasma**
- The **final report** should include quantitative values and an interpretative comment clearly indicating whether results are **positive** or **negative** for LA
- **Comments** such as **borderline** or **dubious LA** are highly discouraged; in such cases the comment should be limited to the following: to be tested again in 1 week

Integrated tests

- Integrated tests include screening and confirmation in a single procedure
- dRVVT or aPTT in the patient's plasma is performed in parallel at low (screen) and high (confirm) PL concentrations
- Although final results may not necessarily require adjunct mixing tests, ***integrated tests without a mixing test should be discouraged***
- Results are interpreted according to the specific cut-off values (% correction or LA ratio)
- Normalisation of results against a PNP run in parallel might be beneficial

LA in acute thromboembolic events

- Consider that the patients might have been treated with
  - full doses of unfractionated heparin
  - vitamin K antagonists (VKA)
- Reactants as fVIII may be increased during acute thromboembolism

LA in long-term VKA therapy

- Perform laboratory procedures 1-2 weeks after discontinuation of treatment or when the INR<1.5
- For INR between 1.5 and < 3.0, a 1:1 dilution of patient plasma and PNP can be considered
- Consider bridging with LMWH (last dose >12h)

Textarin(Taipan)/Ecarin clotting times or integrated tests (i.e.%correction for APTT, SCT and dRVVT at low and high PL concentration) currently not recommended

Final remarks

- An LA result should always be considered in the context of a full laboratory aPL profile comprising aCL and aβ2GPI antibodies ELISAs
- The presence of medium-high titres of aCL and aβ2GPI of the same isotype (most often IgG) is usually found in LA-positive results and identifies patients at high risk for thrombosis
- Isolated LA positivity is found more frequent in subjects without clinical events
- LA tests may be false-positive especially if mild in potency, if found in elderly patients, or if diagnosed for the first time