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Focus
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Circulating anticoagulants

ACC

Circulating anticoagulants (ACC) (Lupus coagulant) were first described in 1952, and then in 1963 their relationship with thrombosis was established. In1975-80 a link with the occurrence of repeat abortions was established. Since 1990 numerous publications have been consecrated to the research of the toxicity mechanisms of lupus anticoagulant/antiphospholipids (aPL) and their link with the predisposition to venous, arterial and capillary thrombosis.

In what circumstances do we screen for lupus anticoagulant?

- In the case of a fortuitous discovery of a lengthening of the aPT with no known etiology;
- In patients who have suffered from arterial or venous thrombosis before the age of 50;
- In the case of thrombosis in an unusual location (mesenteric, cerebral etc) or associated with an auto-immune disease (lupus, rheumatoid arthritis, thrombopenia or haemolytic auto-immune anaemia);
- When faced with obstetric complications.

Definition of antiphospholipids antibodies (aPL)

Antiphospholipid antibodies are a heterogeneous group of antibodies which recognise anionic phospholipids (PL) (cardiolipin, phosphatidylserine) or neutral phospholipids (phosphatidylethanolamine) and / or plasma proteins which bind these phospholipids (such as β2-glycoprotein I or prothrombin).

APL detection

- The lupus type ACC (lupus anticoagulant or LA or antiprothrombinase type ACC) are detected by PL dependent coagulation tests;
- The anticardiolipin antibodies (aCL) and anti-β2- glycoprotein 1 (anti-β2GP1) are detected by ELISA tests.

The antiphospholipids syndrome (APLS)

Diagnosis of APLS = at least 1 clinical criteria + at least 1 biological criteria.

Sapporo clinical criteria

Vascular thrombosis:
- One or several episodes of arterial or venous thrombosis
- regardless of the organ or tissue affected which is confirmed by imagery, Doppler analysis or histopathology, and which must not show vasculitis.

Obstetric symptoms:
- One or several unexplained foetal deaths (›10 weeks) (normal morphology)
- One or more premature births (‹ 34 weeks) due to eclampsia or placental insufficiency,
- Three or more spontaneous abortions (‹ 10 weeks) with no anatomical, hormonal or genetic cause.

Biological criteria

(Revised Sapporo criteria: *S Miyakis et al, J.Thromb Haemost, 2006;4:295-306*)

Persistence › 12 weeks:
- Persistence of a lupus anticoagulant (detected following ISTH recommendations),
- Or a high or average aCL titre: IgG and/or IgM › 40 GPL/MPL (or › 99th percentile of the control tests) by standardised ELISA,
- Or anti-β2GPI IgG and/or IgM, › 99th percentile of the control tests (according to European forum recommendations) by standardised ELISA.

APLS is excluded if more than 5 years have passed between a positive aPL test and the onset of clinical symptoms.

APLS: biological associations

A patient with APLS may have a positive direct Coombs test result, thrombopenia and a lowered C4 fraction result.

New criteria

(Amendments to Sydney 2005) distinguishing different sub-groups of APLS (*Miyakis et al. J Thromb Haemost, February 2006*)

Depending on the type of aPL identified:
- Type I: if a combination of aPL
- Type IIa: isolated LA
- Type IIb: isolated aCL
- Type IIc: isolated anti-β2GPI

Depending on the association with an autoimmune disease (AIDs)
- Primary: without AIDs
- Secondary: with AIDs

Depending on the progressive form
- aPL catastrophic syndrome (CAPS).

Lupus anticoagulant detection by coagulation tests

International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. S Miyakis et al, J.Thromb Haemost,2006;4:295-306.

ISTH recommendations: 4 stages

1. Screening

No single test detects the totality of the LA. Currently it is recommended to use at least two tests, preferably based on different techniques: the aPTT (exploring the intrinsic pathway of coagulation) with a sensitive reagent (neither kaolin nor ellagic acid) and the diluted Russell's Viper Venom Time (dRVVT) (exploring the common pathway). Use of the diluted Thromboplastin Time (DTT) is not recommended.

2. Detection of inhibitory activity

This is a correction test with a mixture of equal parts of patient plasma and normal control plasma, "P+C"
The control plasma to be used must be platelet poor, prepared by double centrifugation or filtration. Ideally one should use a pool of fresh plasma from healthy subjects (n › 20), stored at -80 °C (several months) or at -20 °C (a few weeks), in aliquots of 0.5 ml. It is also possible to use commercial pools, lyophilised or frozen. The test is positive in the absence of correction or insufficient shortening of the lengthening of the screening test. The aPTT is interpreted by the Rosner Index (IR).

$$IR = [(P+C) - C / P] \times 100$$

‹ 13 %: absence of Lupus anticoagulant.
› 13 %: presence of lupus anti coagulant.
The dRVVT result is expressed as R1 ratio (P/C) for screening ≤ 1.20: absence of LA. › 1.20: presence of LA – confirmation necessary)

3. Confirmation of the inhibitor's dependence on phospholipids

This stage allows for the differential diagnosis between the LA and the inhibitors directed against a coagulation factor. The increase in the PL concentration in the medium neutralises the LA effect and reduces the coagulation time, if lengthening is due to a LA. In the aPTT system, it is necessary to calculate Δt after the addition of platelet extracts (Triplett): Staclot PNPR, for phospholipides: Staclot LAR, SCTR.
The dRVVT system is calculated via a normalized ratio: R1/R2 (› 1.20: presence of LA)

4. Exclusion of another coagulation anomaly (specific inhibitors)

Finding lupus anticoagulant does not exclude a coagulation anomaly. This should be eliminated by carrying out quantification analysis of factors VIII, IX, and XI at different dilutions. Typically, in the presence of an LA, the level of all these factors increases by increasing the dilutions up to 1/80th.

APLS pathology: immunological tests

1. Anti-phospholipids antibodies by ELISA

They recognise cardiolipin and anionic PL. The aCL which are independent of any co-factor can be distinguished and detected during infection. The aCL which are dependent on a co-factor are detected during an autoimmune disease and are associated with thrombosis. The detected isotypes are IgG (which are strongly associated with the pathogenesis), and IgM, (which are rare during APLS, and often transitory (infections)); and IgA which are not very informative.
The tests used are aCL ELISA; the results, quantitative, are expressed in the units GPL/MPL in function with the universal 'Harris' standard. There exists a correlation between a raised aCL level and the risk of APLS occurrence.

Haemostasis profile

	Lupus anticoagulant	Anti intrinsic pathway factors	Intrinsic factor deficiency
PT	Normal or moderately decreased	Normal	Normal
aPTT	Increased	Increased	Increased
Rosner index	› 15	› 15	‹ 12
dRVVT (screening/ confirmation)	Positive	Negative	Negative
Intrinsic pathway factors	Normal or decreased ++ factors Correction with plasma dilutions	Decrease in target factor, not corrected with plasma dilution	Decrease in one factor.

2. The other anti-phospholipids antibodies by ELISA

The anti-phosphatidylethanolamine (aPE) antibodies are less well documented. They can be found during APLS without aPL. Their diagnostic interest remains to be demonstrated by multicentric studies.

3. The anti-cofactor protein antibodies.

- Numerous plasma proteins have a role as co-factors for aPL, notably β2GPI or apolipoprotein H. The anti-β2GPI antibodies of the isotypes IgG and IgM are detected by ELISA using purified human β2GP1 as an antigen.

  In practice, it is possible to find serums that are aCL positive / aβ2GP1 negative, which often correspond to the presence of independent β2GP1 aCL in the context of infections or tumours, or aCL directed against other co-factors, that only recognise animal β2GP1; or even serums with negative aCL / positive aβ2GP1 corresponding to antibodies which only recognising human β2GPI, or depend on epitopes situated at the PL binding sites.
- The anti-prothrombin (aPT) antibodies represent a large part of the LA in patients with APLS.

  They are not very specific, and are described in very varied clinical contexts from lupus to infectious episodes. Currently, their detection is of little interest in routine practice.

Situations associated with the presence of aPL (other than APLS)

Autoimmune diseases: Systemic lupus erythematosus, Sharp syndrome, rhumatoid polyarthritis, Gougerot-Sjogren syndrome, scleroderma, IDD, MS, myasthenia and ITP.

Malignant affections: Thymomas, solid tumour cancers, MPS, leukaemia, lymphomas and Waldenström macroglobulinemia.

Infectious diseases: Q fever, syphilis, Lyme's disease, *Mycoplasma* infection, *Chlamydia* infection , *S. Aureus* infection, Streptococcus infection, HIV infection, HCV infection, HBV infection, CMV infection, EBV infection, measles infection, rubella infection, mumps infection, Parvo B19 infection, malaria, and toxoplasmosis etc.

Others: Inducing drugs (phenothiazin, hydantoine, penicillin, hormonal, oestrogeprogesterone drugs procainamide, IFN alpha etc), cirrhosis, terminal renal failure and alcoholism etc.

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