

Advances in Antiphospholipid (Hughes') Syndrome

O Amengual,*^{MD}, T Atsumi,*^{MD, PhD}, M A Khamashta,**^{MD, PhD}, G RV Hughes,***^{MD, FRCP}

Abstract

Fifteen years have passed since Hughes reported the detailed clinical description of antiphospholipid syndrome (APS), and it is now recognised as one of the most common prothrombotic disorders. Its main clinical features are recurrent thrombosis (both venous and arterial), recurrent pregnancy loss and thrombocytopenia associated with the presence of antiphospholipid antibodies (aPLs). aPLs are a heterogeneous group of autoantibodies detected by either clotting or immunological assays. They include lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and antibodies against other phospholipids (PLs). Recently the aPLs family has expanded to include antibodies whose specificity are claimed to be directed not only towards PLs, but also towards plasmaproteins and their complex with PLs. Animal models are providing important new data on clinical and pathogenic aspects of APS. The detection of antibodies against $\beta 2$ glycoprotein I by a simple and rapid enzyme-linked immunosorbent assay (ELISA) may facilitate the recognition of 'pathogenic' aCL in APS. Regarding the treatment of APS, long-term anticoagulation therapy is needed to prevent recurrences.

Ann Acad Med Singapore 1998; 27:61-6

Key words: Anticardiolipin antibodies, $\beta 2$ glycoprotein I, Lupus anticoagulant, Thrombosis

Concept of Antiphospholipid Syndrome (APS)

Phospholipids (PLs) are components of cell membranes. Their function is not only to maintain the structure of the membranes but also to modify the function of proteins present on cell surface. It is known that they play a role in the clotting cascade, especially the initiation and some steps of this cascade depend on the presence of negatively charged PLs. Antiphospholipid antibodies (aPLs) were initially found in patients with systemic lupus erythematosus (SLE). However, a strong association with thrombosis or recurrent pregnancy loss was noted, thus antiphospholipid syndrome (APS) or Hughes syndrome was coined as an independent disease from SLE.^{1,2} Furthermore aPLs were sometimes found in patients with stroke, myocardial infarction, or venous thrombosis without any other underlying systemic disease, and the term primary AIS was introduced to classify these patients.³ The mechanism of thrombosis in patients with AIS remains unknown, but animal models are providing important new data on the pathogenic aspects of APS. The last decade saw a rapid increase in worldwide interest related to this syndrome, firstly because the biological peculiarity of the disease in which autoantibodies cause thrombosis, and secondly because AIS is recognised as one of the most frequent causes of acquired thrombophilia.^{1,4}

Clinical Manifestations in APS

APS is classified into two groups, primary APS and APS secondary to SLE or other connective tissue diseases. However, only a few differences were found in aPL-related complications or in antibody profile in APS patients in the presence or absence of SLE.⁵ Clinical manifestations in APS are heterogeneous, but mostly relate to thrombotic events. Both arterial and venous thrombosis can occur in APS and the recurrent events are common.⁴ Table I shows the summary of 107 consecutive patients visiting the Lupus Clinic at St Thomas' Hospital, London. There were a similar number of patients with primary and secondary APS. The ratio of male to female was relatively higher in the group of primary compared with secondary APS. The onset of APS in our series was around 30 years of age.

Thrombosis

In the arterial circulation, the involvement of intracranial arteries, leading to cerebral infarction or transient ischaemic attacks, is the most frequent. AIS is considered as the most important risk factor for ischaemic cerebral events in young people without congenital atherosclerotic disease. Magnetic resonance imaging (MRI) scans show single or multiple lesions.⁶ In some patients, recurrent multiple small cerebral infarcts lead

* Research fellow

** Director of Research

*** Head of Lupus Unit

Lupus Research Unit, The Rayne Institute

St Thomas' Hospital, London, United Kingdom

Address for Reprints: Dr MA Khamashta, Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, London, SE1 7 EH, United Kingdom.

TABLE I: SUMMARY OF 107 CONSECUTIVE PATIENTS WITH THE ANTIPHOSPHOLIPID SYNDROME (APS), LUPUS CLINIC, ST THOMAS' HOSPITAL, LONDON

Primary APS	61
Secondary APS	46
Female:Male	94: 13
Average age (y)	42
Average duration of disease (y)	9
Arterial thrombosis	53 (50%)
Stroke	37 (35%)
Transient ischaemic attacks	11 (10%)
Coronary heart disease	6 (6%)
Peripheral thrombosis	2 (2%)
Venous thrombosis	52 (49%)
Deep vein thrombosis	35 (33%)
upper limb	3 (3%)
lower limb	32 (30%)
Thrombophlebitis	8 (7%)
Pulmonary embolism	16 (15%)
Recurrent pregnancy loss	33/94 (35%)
Livedo reticularis	31 (29%)
Thrombocytopenia	24 (22%)
Positive lupus anticoagulant	67/88 (76%)
Positive anticardiolipin antibodies	80 (75%)

to dementia and psychiatric symptoms.⁷ Other manifestations of arterial occlusion include ischaemic heart disease, peripheral arterial occlusion, acute abdomen and ischaemic colitis due to mesenteric thrombosis.

In the venous circulation, deep vein thrombosis in the lower extremities is the most common feature, occasionally complicated by pulmonary embolism. Some patients with aPLs also had pulmonary hypertension presumably due to recurrent pulmonary embolism or intravascular thrombosis. In addition, axillary vein thrombosis, central retinal vein thrombosis which may lead to blindness, glomerular vein thrombosis with glomerulosclerosis and adrenal vein thrombosis leading to Addison's disease⁸ have been described in APS. APS is one of the most common causes of Budd-Chiari syndrome associated with hepatic vein thrombosis.⁹ Pathological examination shows bland thrombus without inflammation.^{10,11} Thus, it is unlikely that in AI'S patients, occlusion of blood vessels is due to vasculitis.

Foetal Loss

Recurrent spontaneous pregnancy loss is one of the most consistent complications of APS and aPLs are found in 7% to 20% of women with history of habitual abortions.^{4*} aPL-related foetal losses are strikingly frequent during the second and third trimester. This differs from foetal losses in the non-APS population which usually occur during the first trimester. The mechanism for pregnancy loss is still uncertain but thrombosis of the

placental microvasculature has been suggested.¹³ However, not all placentas examined show signs of placental thrombosis.

Thrombocytopenia

Thrombocytopenia occurs in 20% to 40% patients with APS but severe thrombocytopenia is rare. On the other hand, about 40% of patients diagnosed as idiopathic thrombocytopenic purpura have aPLs [anticardiolipin antibodies (aCL) or lupus anticoagulant (LA)].¹⁴

Other Clinical Features

Epilepsy,¹⁵ chorea¹⁶ and psychiatric disorders are sometimes found in AI'S patients.¹⁷ Transverse myelopathy, although rare, is strongly associated with aPLs.¹⁸ Migraine is common in patients with APS, however prospective studies have not demonstrated a significant association between migraine and aPLs.¹⁹

Heart valve disease, particularly mitral-valve involvement, is associated with aPLs.²⁰ Echocardiographic studies showed that 40% to 77% of patients with aPLs have some valvular involvement such as thickening of the valves or vegetations.²¹ These abnormalities rarely lead to significant valve diseases but may be associated with cerebral vascular events.

Catastrophic AI'S is a rare but life-threatening condition. Patients with catastrophic AI'S develop acute multiple organ failure (notably cerebral and renal), severe thrombocytopenia and adult respiratory distress syndrome. In 30% of cases it may be complicated with disseminated intravascular coagulation (DIC). The prognosis of catastrophic APS is poor with a high fatality rate. The aetiology is unknown but widespread thrombosis has been suggested as the pathogenesis.²²

Diagnosis

In 1990, Harris²³ proposed criteria for the classification of APS. These criteria are shown in Table II. aPLs tests must be positive on at least two occasions more than three months apart.

Anticardiolipin Antibodies

In the early 1980s, assays using cardiolipin (CL) as

TABLE II: PROPOSED CRITERIA FOR THE CLASSIFICATION OF THE ANTIPHOSPHOLIPID SYNDROME*

Clinical	Laboratory
Venous thrombosis	IgG aCL (moderate/ high levels)
Arterial thrombosis	IgM aCL (moderate/ high levels)
Recurrent foetal loss	Positive lupus anticoagulant test
Thrombocytopenia	

aCL: anticardiolipin antibodies

* Patients with this syndrome should have at least one clinical plus one laboratory finding during their disease. The antiphospholipid antibody test must be positive on at least two occasions more than three months apart.

antigen, such as radioimmunoassay²⁴ or enzyme-linked immunosorbent assay (ELISA)²⁵ were established. A strong association was rapidly noticed between the presence of antibodies detected by these assays and thrombosis or pregnancy loss. In 1987, our group²⁶ standardised aCL ELISA and established aCL units to be used internationally. aCL were not only often found in patients with thrombosis but also in patients with infectious diseases, such as malaria, syphilis and acquired immunodeficiency syndrome (AIDS). Furthermore, in SLE patients, aCL were found in 30% to 50% of the cases.^{18,27} Therefore, aCL detected by the conventional aCL assay showed low specificity. It has been demonstrated that aCL has clinical significance when the titres were more than "moderate".²⁸ Thus, high titres of aCL become very specific for the diagnosis of APS but at the expense of a large number of potential true positives. Antibodies against other negatively charged PLs were correlated with aCL suggesting cross reactivity of aCL with other PLs.²⁹

Antigen of aCL: In 1990, three groups³⁰⁻³² reported that aCL associated with AI'S required a plasma protein, β 2 glycoprotein I (β 2GPI), as a cofactor to bind CL in ELISA plates. It was shown that the requirement of β 2GPI for CL binding distinguishes between two types of aCL. One is β 2GPI-independent aCL, mainly found in patients with infectious diseases, which shows high binding to CL in the absence of β 2GPI. The other type of aCL, β 2GPI-dependent aCL, found in patients with autoimmune diseases and associated with thrombotic events, do not bind directly to solid phase CL but to CL- β 2GPI complex.³³ After the discovery of a "cofactor effect" of β 2GPI, some hypotheses regarding the epitope for aCL have been suggested: 1) β 2GPI, 2) β 2GPI-anionic PL complex, 3) anionic PL antigen, and 4) cryptic β 2GPI antigen. Subsequently it was shown that gamma irradiation of polystyrene ELISA plates induces oxygen molecules on the plate surface.³⁴ Antibodies bind to β 2GPI coated onto these oxidised plates, but not to β 2GPI coated on plain polystyrene plates. The binding of antibodies to β 2GPI coated on irradiated plates strongly correlated with the binding obtained by ELISA using β 2GPI-cardiolipin complex as antigen.³⁵ Furthermore, experimental studies showed that monoclonal aCL derived from lupus-prone mice or from APS patients bound to β 2GPI coated onto irradiated plates.³⁶ Thus, aCL found in AI'S patients bind to β 2GPI whose conformational change is induced by irradiated polystyrene surface or through interaction with negatively charged PLs, allowing the exposure of the cryptic epitope of β 2GPI.

New assays to detect aCL: Recently a new assay to directly measure antibodies to human β 2GPI in the absence of CL has been established and its clinical significance is being studied. In a large cohort of SLE patients, IgG anti β 2GPI antibodies correlated with a

history of thrombosis.³⁵ This method not only allowed differentiation between aCL associated with APS from aCL found in infectious diseases but also from "non-specific" aCL often found in SLE.²⁹ However, the sensitivity of IgG aCL conventional assay was 70.4% while the sensitivity of anti β 2GPI antibodies assay was only 53%, implying that anti β 2GPI assay is not suitable for the screening of AI'S because of its lower sensitivity. On the other hand, it has been reported that anti β 2GPI antibodies could occasionally be missed by conventional aCL assay.³⁷ There are two possible explanations: 1) the epitope on β 2GPI is not available when β 2GPI binds to CL, and 2) these anti β 2GPI antibodies are species specific (i.e. recognising human, but not bovine β 2GPI).³⁸

Lupus Anticoagulant

LA is defined as immunoglobulins which interfere with some steps of the *in vitro* PL-dependent coagulation reaction [i.e. activated partial thromboplastin time (APTT), kaolin clotting time (KCT) and dilute Russell viper venom time (dRVVT)]. Table III shows the method for the detection of LA widely being practised. Due to its high heterogeneity, no single test will successfully identify all LA. Guidelines for testing LA have been proposed.³⁹ A three-step approach has been recommended: 1) screening test to see if PL-dependent coagulation test(s) are prolonged, 2) mixing test to demonstrate that the prolongation of the clotting time is caused by an inhibitor present in plasma rather than by a factor deficiency, and 3) neutralisation test by damaged platelets or by PLs to characterise the inhibitor as aPL. In patients receiving heparin or oral anticoagulation, the determination of LA is difficult.

Cofactor of LA: It had been suggested that LA activity requires plasma cofactors. Some LA plasma requires β 2GPI for LA activity⁴⁰ and a range of mouse monoclonal anti β 2GPI antibody showed LA activity.⁴¹ Recently, it was reported that β 2GPI and rabbit anti human β 2GPI antibody inhibited synchronously the activation of fac-

TABLE III: DETECTION OF LUPUS ANTICOAGULANT

Screening test

Activated partial thromboplastin time (APTT)
Kaolin clotting time (KCT)
Dilute Russell viper venom time (dRVVT)
Dilute tissue thromboplastin inhibitor test (TTI)
Textarin clotting time
Plasma clotting time (PCT)

Confirmatory test

Decreased phospholipids: TTI, dRVVT
Increased phospholipids: High rabbit brain phospholipid
Altered configuration of phospholipid: Hexagonal (II) phase phosphatidylethanolamine
Platelet neutralisation procedure (PNP)

tor XII, implying that β 2GPI-anti β 2GPI complex is one of the LA.⁴² However, not all of anti β 2GPI antibodies have LA activity. Bevers et al⁴³ suggested that LA recognises prothrombin-PL complex. Therefore, PL-dependent coagulation reaction may be impeded at least by two types of LA, one is β 2GPI-dependent and the other prothrombin-dependent.

Expanded aPLs family

In the last few years interest has been focused on antibodies against PL-binding proteins rather than in antibodies against PLs themselves.⁴⁴ As described above, prothrombin is one of the cofactors of LA. Plasma concentration of prothrombin is similar to that of β 2GPI (≈ 150 μ g/ml). Antibodies against prothrombin can be detected by ELISA when prothrombin is coated on irradiated plates or bound to phosphatidylserine, but the clinical significance of these antibodies has not been fully investigated.

Protein C and protein S are vitamin K dependent anticoagulant factors. They bear Gla-domain as a PL binding site. In some AI'S patients low plasma levels of protein C or protein S are found (especially free form of protein S which has anticoagulant activity). Antibodies against lipid-bound protein C and protein S have been described in some patients with APS.⁴⁵

Annexin V, one member of the annexin family, has high affinity for anionic PLs in a calcium dependent fashion. This protein behaves as a potent natural anticoagulant. Antibodies against annexin V have been investigated as a possible explanation of recurrent abortions or foetal death.⁴⁶

Mechanism of Thrombosis

Great interest has centred on the role of PL-binding proteins as a clue of the mechanism of thrombosis in APS. The better characterised are β 2GPI and prothrombin. β 2GPI binds to negatively charged PLs and regulates PL-dependent coagulation reactions, thus has both procoagulant and anticoagulant properties. Table IV shows the functions of β 2GPI *in vitro*. In APS patients' plasma, the concentration of β 2GPI is normal or rather increased. Furthermore, congenital deficiency of this protein is not a clear risk factor for thrombosis. Animal models proved the importance of the role of β 2GPI in APS. Animals immunised with human β 2GPI produce both anti human β 2GPI and non-specific aPLs (β 2GPI-independent aCL) and develop intrauterine foetal death or thrombocytopenia, similar to human APS. It had been hypothesised that these antibodies bind to endothelial cells or platelets via PL-binding proteins and induce the production of procoagulant substances by these cells. As a consequence of initial damage, anionic PLs are exposed on the cell surface. These PLs are covered by PL-binding proteins, such as β 2GPI or prothrombin. If aPLs

TABLE IV: PROPERTIES OF β 2 GLYCOPROTEIN I IN VITRO

Anticoagulant
1. Inhibition of prothrombinase activity (thrombin formation)
2. Inhibition of factor X activation
3. Inhibition of factor XII activation
4. Inhibition of platelet aggregation
Procoagulant
1. Inhibition of protein C activation
2. Inhibition of inactivation of activated Factor V by activated protein C

are present against such surface-bound proteins, aPLs may bind them and subsequently induce the production of a procoagulant substances such as tissue factor, plasminogen activator inhibitor (PAI-1), or adhesion molecules.⁴⁷ Furthermore, β 2GPI binds to oxidised-LDL, and monoclonal aCL increases the uptake of oxidised-LDL by macrophage-like cell line.⁴⁸ These findings suggest that aPLs are involved in the progression of atherosclerosis, particularly interesting in relation to the pathogenesis of arterial thrombosis.

Treatment

Despite the enormous amount of work focused on the pathogenesis and clinical manifestations of APS, there has been surprisingly little published on the management of thrombosis associated with APS. One of the most practical advances in the management of SLE has been the recognition that a significant number of patients suffer from recurrent thrombotic episodes, strongly associated with aPLs.

Asymptomatic Individuals

The controversy concerning whether or not prophylactic treatment for asymptomatic individuals with aPLs remains unresolved.⁴⁹ In our daily practice, we recommend that patients with persistently positive lupus anticoagulant test results and/or moderate to high IgG aCL levels and no history of thrombosis may be treated with low-dose aspirin (75 mg/day). aCL test is positive at low titres in about 2% of healthy obstetric population and such finding does not appear to be associated with an adverse outcome. Because the risk is relatively low for primiparas, even if they have high aPLs titres, the recommended prophylactic treatment is either close observation only or low-dose aspirin (75 mg/day).⁵⁰

Thrombosis

There is now strong evidence that the thrombosis in APS tends to recur, thus requires long-term, possibly lifelong prophylactic therapy. We assessed our experience of the management of aPLs-associated thrombosis in 147 patients over a 10-year period and found that long-term and intensive oral anticoagulation (international normalised ratio ≥ 3) was the most effective therapeutic option in the secondary prevention of venous and

arterial thrombosis.⁵¹ No evidence was found that low-dose aspirin (75 mg/day) alone prevented recurrence of thrombosis in our patients. The use of steroids and immunosuppressive agents to reduce antibody levels has not proved beneficial in the long-term.^{51,52} Some experimental treatments with fibrinolytic substances⁵³ or prostaglandin analogues⁵⁴ have been successful in selected patients with APS.

The recognition of catastrophic AFS requires immediate management in an intensive care unit and treatment of the multiple organ failure together with intensive anti-thrombotic treatment and plasmapheresis.⁵⁵

Recurrent Pregnancy Loss

Recent data from our pregnancy clinic show that the greatest impact on foetal outcome in patients with SLE and AFS has been the regularity and quality of obstetric care, judicious monitoring, and timely intervention.⁵⁶ Low-dose aspirin (75 mg/day) is effective in preventing pregnancy loss, and low molecular weight heparin (5000 to 10 000 IU/day) appears to prevent recurrent thromboembolic disease in pregnant woman at high risk.⁵⁷ The use of corticosteroids and gammaglobulins is still controversial.

Conclusion

To define the role of aPLs in the pathogenesis of thrombosis requires further and intensive investigations. The availability of more accurate diagnostic techniques may facilitate the recognition of pathogenic aPLs. This would be an important step in the management of patients with AFS and could lead to more precise treatment.

REFERENCES

- Hughes G R V. The antiphospholipid syndrome: ten years on. *Lancet* 1993; 342:341-4.
- Khamashta M A, Asherson R A. Hughes syndrome: antiphospholipid antibodies move closer to thrombosis in 1994. *Br J Rheumatol* 1995; 34:493-4.
- Asherson R A. A "primary" antiphospholipid syndrome. *J Rheumatol* 1988; 15:1742-6.
- Khamashta M A, Hughes G R V. Antiphospholipid antibodies and antiphospholipid syndrome. *Curr Opin Rheumatol* 1995; 7:389-94.
- Vianna J L, Khamashta M A, Ordi-Ros J, Font J, Cervera R, Lopez-Soto A, et al. Comparison of the primary and secondary antiphospholipid syndrome: A European multicenter study of 114 patients. *Am J Med* 1994; 96:3-9.
- Stimmler M M, Coletti P M, Quismorio F P. Magnetic resonance imaging of the brain in neuropsychiatric systemic lupus erythematosus. *Semin Arthritis Rheum* 1993; 22:335-49.
- Toubi E, Khamashta M A, Panarra A, Hughes G R V. Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. *Am J Med* 1995; 99:397-401.
- Le T H, Wechsler B, Piette J C, Doumith R, Bellin M F, Papo T, et al. The antiphospholipid syndrome: A new cause of bilateral adrenal haemorrhage: four cases. *Presse Med* 1993; 22:249-54.
- Pelletier S, Landi B, Piette J C, Ekert I, Coutellier A, Desmoulins C, et al. Antiphospholipid syndrome as the second cause of non-tumorous Budd-Chiari syndrome. *J Hepatol* 1994; 21:76-80.
- Hughson M D, McCarty-Farid G A, Brumback R A. Spectrum of vascular pathology affecting patients with the antiphospholipid syndrome. *Hum Pathol* 1995; 26:716-24.
- Ford S E, Kennedy L, Ford P M. Clinicopathologic correlations of antiphospholipid antibodies: an autopsy study. *Arch Pathol Lab Med* 1994; 118:491-5.
- Branch D W, Silver R M. Criteria for antiphospholipid syndrome: early pregnancy loss, fetal loss, or recurrent pregnancy loss? *Lupus* 1996; 5:409-13.
- Branch D W. Thoughts on the mechanism of pregnancy loss associated with the antiphospholipid syndrome. *Lupus* 1994; 3:275-80.
- Stasi R, Stipa E, Masi M, Oliva F, Sciarra A, Perrotti A, et al. Prevalence and clinical significance of elevated antiphospholipid antibodies in patients with idiopathic thrombocytopenic purpura. *Blood* 1994; 84:4203-8.
- Herranz M T, Rivier G, Khamashta M A, Blaser K U, Hughes G R V. Association between antiphospholipid antibodies and epilepsy in patients with systemic lupus erythematosus. *Arthritis Rheum* 1994; 37:568-71.
- Asherson R A, Derksen R, Harris E N, Hughes G R V. Chorea in systemic lupus and lupus-like disease: association with antiphospholipid antibodies. *Semin Arthritis Rheum* 1987; 16:253-9.
- Levine S R, Brey R L. Neurological aspects of antiphospholipid antibody syndrome. *Lupus* 1996; 5:347-53.
- Alarcon-Segovia D, Deleze M, Oria C V, Sanchez-Guerrero J, Gomez-Pacheco L, Gabiedes J, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine (Baltimore)* 1989; 68:353-65.
- Tsakiris D A, Kappos L, Reber G, Marber G A, Le Floch-Rohr J, Roux E, et al. Lack of association between antiphospholipid antibodies and migraine. *Thromb Haemostasis* 1993; 69:415-7.
- Khamashta M A, Cervera R, Asherson R A, Font J, Gil A, Coltart D J, et al. Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus. *Lancet* 1990; 335:1541-4.
- Hojnik M, George J, Ziporen L, Shoenfeld Y. Heart valve involvement (Libman-Sacks endocarditis) in the antiphospholipid syndrome. *Circulation* 1996; 93:1579-87.
- Asherson R A. The catastrophic antiphospholipid syndrome. *J Rheumatol* 1992; 19:508-12.
- Harris E N. Antiphospholipid antibodies. *Br J Haematol* 1990; 74:1-9.
- Harris E N, Gharavi A E, Boey M L, Patel B M, Mackworth-Young C G, Loizou S, et al. Anti-cardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983; ii:1211-4.
- Koike T, Sueishi M, Funaki H, Tomioka H, Yoshida S. Antiphospholipid antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1984; 56:193-9.
- Harris E N, Gharavi A E, Patel B M, Hughes G R V. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. *Clin Exp Immunol* 1987; 68:215-22.
- Cervera R, Khamashta M A, Font J, Sebastiani G D, Gil A, Lavilla I, et al. Systemic lupus erythematosus: Clinical and immunological patterns of disease expression in a cohort of 1000 patients. *Medicine (Baltimore)* 1993; 72:113-24.
- Harris E N, Chan J K H, Asherson R A, Aber V R, Gharavi A E, Hughes G R V. Thrombosis, recurrent fetal loss, and thrombocytopenia. Predictive value of the anticardiolipin antibody test. *Arch Intern Med* 1986; 146:2153-6.
- Amengual O, Atsumi T, Khamashta M A, Koike T, Hughes G R V. Specificity of ELISA for antibody to β_2 -glycoprotein I in patients with antiphospholipid syndrome. *Br J Rheumatol* 1996; 35:1239-43.
- McNeil H P, Simpson R J, Chesterman C N, Krilis S A. Anti-phospholipid antibodies are directed against a complex antigen that induces a lipid-binding inhibitor of coagulation: β_2 -glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci USA* 1990; 87:4120-4.

31. Galli M, Comfurius I, Maassen C, Hemker C, DeBaets M H, van Bredavriesman P J C, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 1990; 335:952-3.
32. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. *Lancet* 1990; 336:177-8.
33. Arvieux J, Roussel B, Jacob M C, Colomb M G. Measurement of antiphospholipid antibodies by ELISA using β 2-glycoprotein I as an antigen. *J Immunol Methods* 1991; 143:223-9.
34. Matsuura E, Igarashi Y, Yasuda T, Triplett D A, Koike T. Anticardiolipin antibodies recognize β 2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* 1994; 179:457-62.
35. Tsutsumi A, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Kobayashi S, et al. Antibodies to β 2-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis Rheum* 1996; 39:1466-74.
36. Ichikawa K, Khamashta M, Koike T, Matsuura E, Hughes G R V. Reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syndrome to β 2-glycoprotein I. *Arthritis Rheum* 1994; 37:1453-61.
37. Cabral A R, Amigo M C, Cabiedes J, Alarcón-Segovia D. The antiphospholipid/cofactor syndromes: a primary variant with antibodies to β 2-glycoprotein I but no antibodies detectable in standard antiphospholipid assay. *Am J Med* 1996; 101:472-81.
38. Roubey R A S. Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum* 1996; 39:1444-54.
39. Triplett D A. Assays for detection of antiphospholipid antibodies. *Lupus* 1994; 3:281-7.
40. Roubey R A S, Pratt C W, Buyon J P, Winfield J B. Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent upon β 2 glycoprotein I. *J Clin Invest* 1992; 90:1100-4.
41. Arvieux J, Pouzol I, Roussel B, Jacob M C, Colomb M G. Lupus-like anticoagulant properties of murine monoclonal antibodies to β 2-glycoprotein I. *Br J Haematol* 1992; 81:568-73.
42. Schousboe I, Rasmussen M S. Synchronized inhibition of the phospholipid mediated autoactivation of factor XII in plasma by β 2-glycoprotein I and anti- β 2-glycoprotein I. *Thromb Haemost* 1995; 73:798-804.
43. Bevers E M, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost* 1991; 66:629-32.
44. Roubey R A S. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" antibodies. *Blood* 1994; 84:2854-67.
45. Oosting J D, Derksen R H W M, Bobbink I W G, Hackeng T M, Bouma B N, de Groot P G. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: An explanation for their pathogenic mechanism? *Blood* 1993; 81:2618-25.
46. Kaburaki J, Kuwana M, Yamamoto M, Kawai S, Ikeda Y. Clinical significance of anti-annexin antibodies in patients with systemic lupus erythematosus. *Am J Hematol* 1997; 54:209-13.
47. Arnout J. The pathogenesis of the antiphospholipid syndrome: a hypothesis based on parallelisms with heparin-induced thrombocytopenia. *Thromb Haemost* 1996; 75:536-41.
48. Hasunuma Y, Matsuura E, Makita Z, Katahira T, Nishi S, Koike T. Involvement of β 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin Exp Immunol* 1997; 107:569-73.
49. Khamashta M A. Management of thrombosis in the antiphospholipid syndrome. *Lupus* 1996; 5:463-6.
50. Lockshin M D. Which patients with antiphospholipid antibody should be treated and how? *Rheum Dis Clin North Am* 1993; 19:235-47.
51. Khamashta M A, Cuadrado M A, Mujic F, Taub N A, Hunt B J, Hughes G R V. The management of thrombosis in the antiphospholipid-antibody syndrome. *N Engl J Med* 1995; 332:993-7.
52. Asherson R A, Baguley E, Pal C, Hughes G R V. Antiphospholipid syndrome: five year follow up. *Ann Rheum Dis* 1991; 50:805-10.
53. Gertner E, Lie J Y. Systemic therapy with fibrinolytic agents and heparin for recalcitrant non-healing cutaneous ulcer in the antiphospholipid syndrome. *J Rheumatol* 1994; 21:2159-61.
54. Zahavi J, Charach G, Schafer R, Toeg A, Zahavi M. Ischaemic necrotic toes associated with antiphospholipid syndrome and treated with iloprost. *Lancet* 1993; 342:862.
55. Asherson R A, Cervera R. "Primary", "secondary" and other variants of the antiphospholipid syndrome. *Lupus* 1994; 3:293-8.
56. Lima F, Khamashta M A, Buchanan N M N, Kerslake S, Hunt B J, Hughes G R V. A study of sixty pregnancies in patients with the antiphospholipid syndrome. *Clin Exp Rheumatol* 1996; 14:131-8.
57. Hunt B J, Doughty H, Majumdar G, Coppelstone A, Kerslake S, Buchanan N, et al. Thromboprophylaxis with low molecular heparin (fragmin) in high risk pregnancies. *Thromb Haemost* 1997; 77:39-43.