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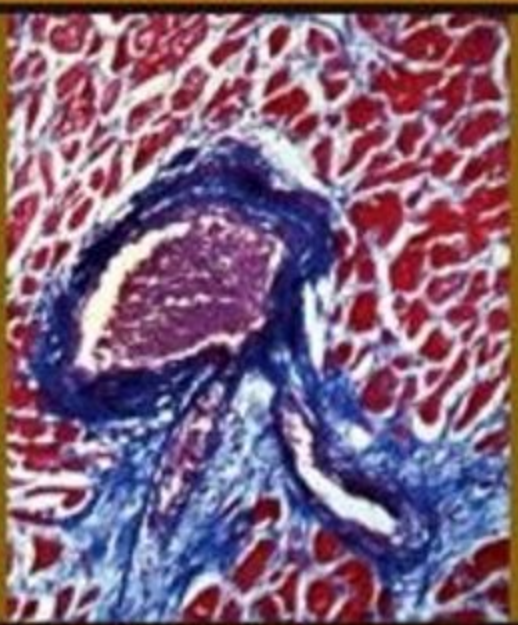

Antiphospholipid Syndrome in Systemic Autoimmune Diseases

Edited by
Ricard Cervera, Joan Carles Reverter & Munther Khamashta

Handbook of Systemic Autoimmune Diseases

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Antiphospholipid Syndrome in Systemic Autoimmune Diseases

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Dedication

The authors wish to dedicate this book to the memory of Ronald A. Asherson, series editor of the *Handbook of Systemic Autoimmune Diseases*, who passed away while this final volume was in preparation. Dr. Asherson spent many years of his life bringing together dozens of specialists from different parts of the world and from different fields of medicine in order to improve our understanding of these diseases, especially of the antiphospholipid syndrome, the topic of this volume.

Ricard Cervera
Joan Carles Reverter
Munther A. Khamashta

Preface

In the 25 years since the original description of the antiphospholipid syndrome, there have been many notable advances in our ability to recognize both the clinical and the underlying aspects of the condition.

Now realized to be a major cause of common conditions, including stroke, heart attack, miscarriage, epilepsy, and memory loss, the syndrome is at last gaining recognition in all branches of medicine, from obstetrics to cardiology, from psychiatry to orthopedics.

The 1983 description of "the anticardiolipin syndrome" included the basic clinical features, such as the tendency for arterial as well as venous thrombosis, the major link with recurrent miscarriage, the clinical distinction from lupus and the importance of neurological features such as memory loss, migraine, stroke, chorea, and myelopathy. Other clinical observations at that time included thrombocytopenia, livedo reticularis, labile hypertension, Budd–Chiari syndrome, renal thrombosis, and pulmonary hypertension.

The ensuing 25 years have witnessed two clinical developments—first the growth of the syndrome into a fully-fledged systemic disease and, second, the recognition of its importance across the broad canvas of medicine. Examples of the latter include its link with up to 1 in 5 cases of young stroke (and possibly 1 in 5 cases of young myocardial infarction), 1 in 5 deep vein thromboses, and 1 in 5 cases of recurrent miscarriage. It is an important cause of teenage epilepsy and a differential diagnosis in cases of multiple sclerosis and Alzheimer's. Migraine is a particularly common feature—indeed, in this clinician's opinion, the syndrome may well prove to be the "missing link" between migraine and stroke.

The science has also moved on, bringing with it the recognition of the importance of phospholipid-binding proteins, the mechanisms of thrombosis, data from animal models, the genetics, the links to atheroma, and the epidemiology. One area which possibly lags behind is that of blood testing. In the quarter of a century since our publication of immunoassays for antiphospholipid antibodies, significant variation still exists between different assays, especially with the proliferation of numerous "kits."

This volume highlights for me the overwhelming sense of collaboration there has been over the course of this story. Many of the authors (Ricard Cervera, Nigel Harris, Angela Tincani, Ron Asherson (sadly deceased), Munther Khamashta, Guillermo Ruiz-Irastorza, Robert Roubey) were fellows in my unit, as was the late genius Aziz Gharavi, whose early contributions were pivotal.

I am proud to have been associated with these colleagues and with the antiphospholipid syndrome, and am truly honored by those friends who gave the eponymous name "Hughes syndrome" to the disease.

In my years of study in medicine and in rheumatology I have witnessed no other topic in which such a strong, friendly, and, indeed, successful collaborative approach has characterized progress. One perfect example of this is Ricard Cervera's ongoing "Euro-phospholipid Project."

This volume, bringing together many of those experts and collaborators, represents another advance in our understanding and our teaching of this major disease of our time.

Graham Hughes

Series Editor

In Memoriam—Ronald A. Asherson (1934–2008)

Dr. Ronald A. Asherson, the series editor of these *Handbooks of Systemic Autoimmune Diseases*, died in May, 2008 at the age of 73 in Johannesburg, South Africa. The Editors of this volume remain profoundly affected by this sudden loss, but wish to pay tribute to his memory and the immense human and professional legacy that he leaves. Dr. Asherson was an internationally renowned authority in the field of autoimmune diseases, especially the antiphospholipid syndrome (APS). He made numerous contributions to the recognition and characterization of the many clinical manifestations associated with the antiphospholipid antibodies, including the original description of the most severe variant of the syndrome—the ''catastrophic'' APS. He was also a wonderful speaker, who influenced many scientists and physicians to work and do research on this field of medicine, and a great traveler, who collected interesting cases from all over the world for medical journal reports, as well as intellectual and cultural experiences and, more importantly, good and respectful friends.

Ron Asherson was born in Cape Town, South Africa, in 1934, and qualified in medicine at the university of this city in 1957. He moved to England in 1960 and become house officer to Professor Sir Christopher C. Booth at the Hammersmith Hospital, London. In 1961, he accepted a fellowship at the Columbia Presbyterian and Francis Delafield Hospitals in New York, returning to South Africa to become senior registrar at the Groote Schuur Hospital in Cape Town from 1961 to 1964. After 10 years as a clinical tutor in its Department of Medicine, he went back to the United States and was appointed as assistant clinical professor of medicine at the New York Hospital-Cornell Medical Center under Professor Henry O. Heineman. From 1981 to 1985, he was associated with the Rheumatology Department at the Royal Postgraduate Medical School of London (Hammersmith Hospital), working under Dr. Graham R.V. Hughes. It was at that time that he developed his interest in systemic autoimmune diseases and antiphospholipid antibodies.

In 1985, he moved with Dr. Hughes to the Rayne Institute at St. Thomas' Hospital to create the currently worldwide renowned Lupus Unit. In 1991 he took a sabbatical at St. Luke's Roosevelt Hospital Center in New York, working with Professor Robert Lahita. In 1992, he returned to South Africa to become principal scientific officer at the Rheumatic Disease Unit, Groote Schuur Hospital in Cape Town, and later associate professor at the Division of Immunology, School of Pathology, University of the Witwatersrand, Johannesburg. He was also visiting professor at the Department of Autoimmune Diseases, Hospital Clínic, Barcelona, Catalonia, Spain.

The research output of Ron Asherson has been prodigious, with a total of over 500 scientific articles published, more than 100 chapters in leading textbooks on rheumatology and internal medicine, several books edited—including *Phospholipid Binding Antibodies*, *Vascular Manifestations of Systemic Autoimmune Diseases*, and two editions of *The Antiphospholipid Syndrome*, as well as series editor of the 10 volumes *Handbooks of Systemic Autoimmune Diseases.* He was on the Editorial Boards of several international journals.

Among other awards, in 1992, he was the co-winner of the European League Against Rheumatism (EULAR) Prize and in 1993 was part of Graham Hughes' team awarded the International League Against Rheumatism (ILAR) Prize, both for research on antiphospholipid antibodies. In 1994, he was elected Fellow of the Royal College of Physicians (FRCP) of London. In 2001, he was awarded as *Doctor Honoris Causa* by the University of Pleven in Bulgaria. He was also awarded the Gold medal and Citation for his contributions to research by the Comenius University in Bratislava in April, 2008.

Until the end, Ron continued visiting patients in his private practice clinic, writing papers, planning new projects—and receiving lots of love and respect. His life and the manner of his death will remain a lasting memory to all who had the great fortune to know him.

Ricard Cervera
Joan Carles Reverter
Munther A. Khamashta
Editors

Volume Editors

Ricard Cervera

Dr. Ricard Cervera, is senior consultant and head at the Department of Autoimmune Diseases, Hospital Clinic, Barcelona, Director of the Research Group on Systemic Autoimmune Diseases at the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) of Barcelona, and associate professor at the Department of Medicine, Universitat de Barcelona, Barcelona, Catalonia, Spain.

Dr. Cervera qualified in medicine in 1983 from the Universitat de Barcelona and in 1988 obtained his PhD degree for his thesis on anticardiolipin antibodies. His post-doctoral experience included 2 years at the Lupus Research Unit, The Rayne Institute, St. Thomas' Hospital, London. In 1995, together with Drs Miguel Ingelmo and Josep Font he created the Department of Autoimmune Diseases of the Hospital Clínic de Barcelona, a pioneering center in Europe devoted to developing high-quality clinical, academic, and research work on these conditions.

Dr. Cervera is a member of the Catalan, Spanish, and International Societies of Internal Medicine and the Spanish Society of Rheumatology, Fellow of the Royal College of Physicians (FRCP) of London, and honorary member of the Argentinian, Mexican, Peruvian, Equatorian, and Slovak Societies of Rheumatology. He is coordinator of the "Euro-lupus" project and of the European Working Party on Systemic Lupus Erythematosus and has organized 10 international workshops of this working party in several cities of Europe as well as several international symposia and congresses on autoimmune diseases, including co-chairing the "6th International Congress on Autoimmunity" in 2008.

Among other awards, he has received the Spanish Society of Internal Medicine Award for research on antibodies to endothelial cells, the Fernández-Cruz Award from the Rhône-Poulenc Farma Foundation, the Juan Vivancos Award from the Consorci d'Hospitals de Barcelona, and the Prizes of the "5th European Conference on Systemic Lupus Erythematosus," EULAR 2003, and EULAR 2005. He has been the recipient of research grants from the Spanish Departments of Health and Education and Science, British Council and the European Commission.

Dr. Cervera has presented over 300 invited lectures and has published more than 500 scientific papers, including original articles in *The Lancet*, *Arthritis and Rheumatism*, *American Journal of Medicine* and *Medicine (Baltimore)*. His academic activities include invited professorships in several European and Latin-American universities. He is co-editor of 25 books, including *The Antiphospholipid Syndrome*, *Vascular Manifestations of Systemic Autoimmune Diseases*, and *Diagnostic Criteria of Autoimmune Diseases*, among others. He is also member of the Editorial Board of 10 medical journals and Editor of the scientific journals *Current Medical Literature—Rheumatology*, *Annals of Autoimmunity* (e-journal), and *Rheuma21st* (e-journal). His current major research interest includes clinical and epidemiological aspects of systemic autoimmune diseases, particularly systemic lupus erythematosus and the antiphospholipid syndrome, with special focus on its "catastrophic" variant.

Dr. Cervera is married to Carme and has two daughters, Marta and Laura. He is also enthusiastically involved in social and cultural activities and is currently the chair of the Association of Friends of the Museum of Gavà (Barcelona) and Secretary General of the Catalan Federation of Friends of Museums.

Joan Carles Reverter

Dr. Joan Carles Reverter was born in Badalona (Barcelona, Catalonia, Spain) in 1959. He graduated in medicine and surgery at the Medical School of the University of Barcelona in 1982 and also obtained his PhD in the same university. He specialized in hematology and hemotherapy at the Hospital Clínic of Barcelona and trained in hemostasis at Mount Sinai Hospital in New York. Today he is the head of the

hemostasis section (Department of Hemostasis and Hemotherapy) in the Hospital Clinic of Barcelona. He has published more than 150 original papers in international journals and has received more than 25 competitive research grants. At present he is the secretary of the Spanish Society of Thrombosis and Hemostasis, the president of the Standardization Committee of the Spanish Association of Hematology and Hemotherapy, and the president of the Catalonian Society of Hematology and Hemotherapy.

Munther Khamashta

Munther Khamashta is senior lecturer/consultant physician and director of lupus research in the Lupus Unit at St Thomas' Hospital, London.

His major interest is lupus and connective tissue diseases in general, with a special interest in pregnancy and antiphospholipid syndrome. He has published extensively in this field (more than 400 original papers) and has also published several books, his most recent one in 2006, the second edition of his textbook *Hughes Syndrome: Antiphospholipid Syndrome*.

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Handbook of Systemic Autoimmune Diseases, Volume 10
Antiphospholipid Syndrome in Systemic Autoimmune Diseases
Ricard Cervera, Joan Carles Reverter and Munther Khamashta, editors

CHAPTER 1

History, Classification, and Subsets of the Antiphospholipid Syndrome

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1. Introduction

The antiphospholipid syndrome (APS) is defined by the occurrence of venous and arterial thromboses, often multiple, and pregnancy morbidity (abortions, fetal deaths, premature births), in the presence of antiphospholipid antibodies (aPL), namely lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), or anti-β_2 glycoprotein I (β_2GPI) antibodies. The APS can be found in patients having neither clinical nor laboratory evidence of another definable condition (primary APS) or it may be associated with other diseases, mainly systemic lupus erythematosus (SLE), but occasionally with other autoimmune conditions, infections, drugs, and malignancies. Rapid chronological occlusive events, occurring over days to weeks, have been termed the catastrophic APS. Other postulated APS subsets include the microangiopathic and the seronegative APS.

2. Historical perspective

Wassermann et al. (1906) discovered "reagin," an antibody reacting with an antigen located in alcohol extracts of liver from a fetus with congenital syphilis. Pangborn (1941) showed that this antigen was a phospholipid which was named cardiolipin. The subsequent use of cardiolipin, together with phosphatidylcholine and cholesterol, led to the development of various precipitation complement fixation techniques to detect reagin. During World War II, individuals with positive serologic test results for syphilis were identified, but they had no clinical evidence of the disease. It became apparent that false-positive serologic test results for syphilis might occur occasionally, usually as a result of an acute infection such as malaria or endocarditis. In 1955 it was shown that patients with endocarditis had a high incidence of autoimmune disorders, especially SLE (Moore and Lutz, 1955).

In 1952, an in vitro inhibitor of coagulation was found in two patients with SLE. This inhibitor was frequently associated with false-positive serologic test results for syphilis and could be absorbed from plasma by phospholipids (Conley and Hartman, 1952). In 1972, it was named lupus anticoagulant, although later it was disclosed that 50% of patients with this serologic abnormality do not have SLE and, although the antibody acts as an anticoagulant in vitro, in vivo it is mainly associated with thrombotic events and less frequently with hemorrhage (Feinstein and Rapaport, 1972).

In the early 1980s, studies carried out at London's Hammersmith Hospital by Graham R.V. Hughes and colleagues led to the

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development of solid-phase immunoassays to detect aCL (Harris et al., 1983). A high correlation between the IgG isotype of aCL and clinical thrombosis was documented and a close relationship between these antibodies and the presence of the LAC was also demonstrated (Harris et al., 1986). These findings led to the recognition of the so-called anticardiolipin syndrome, which was later more correctly named the APS or Hughes syndrome (Hughes, 1983). In 1987, Dr Hughes' group recognized that some individuals without lupus or antinuclear antibodies (ANA) developed the syndrome. These patients were classified as having a primary APS (Asherson, 1988).

A major advance came in the early 1990s with the simultaneous recognition by three different groups that the aPL required a plasma protein "cofactor" to bind cardiolipin on ELISA plates. β_2GPI was identified as this cofactor. Since then, a number of "cofactors," including prothrombin, have been described. In 1992, Asherson described a subset of patients with a widespread coagulopathy affecting predominantly small vessels that led to rapid multiorgan failure. This dramatic clinical situation was termed catastrophic APS (Asherson, 1992).

Table 1
Preliminary classification criteria for APS (Sapporo criteria)

Clinical criteria

1. Vascular thrombosis
   One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by imaging or Doppler studies or histopathology, with the exception of superficial venous thrombosis. For histopathological confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall
2. Pregnancy morbidity
   (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10^{th} week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus
   (b) One or more premature births of a morphologically normal neonate at or before the 34^{th} week of gestation because of severe preeclampsia or eclampsia, or severe placental insufficiency or
   (c) Three or more unexplained consecutive spontaneous abortions before the 10^{th} week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded

Laboratory criteria

1. aCL of IgG and/or IgM isotype in blood, present in medium or high titers, on 2 or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent assay (ELISA) for β_2-glycoprotein-I-dependent anticardiolipin antibodies
2. LAC present in plasma, on 2 or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis

3. Classification of the antiphospholipid syndrome

In 1999, a preliminary classification criteria was established after an expert workshop held in Sapporo, Japan (Wilson et al., 1999) (Table 1). The need for consensus APS criteria was highlighted by the diversity of clinical and basic science disciplines that contribute to the diagnosis and treatment of APS and by the lack of uniformity in previous proposed criteria for APS. More recently, another workshop was held in Sydney, Australia, in which experts proposed some modifications to previous criteria, such as the inclusion of anti-β_2GPI antibodies. Although no new clinical criteria were added, some particular features were remarked on, such as associated APS features, including cardiac valve involvement, livedo reticularis, thrombocytopenia, APS nephropathy, and non-thrombotic central nervous system manifestations (i.e. cognitive dysfunction) (Table 2) (Miyakis et al., 2006).

APS can present in different scenarios, such as asymptomatic "carrier" patients for aPL, "classical" APS with recurrent venous and/or arterial thrombosis, APS affecting otherwise healthy women with recurrent pregnancy loss, aPL-positivity with non-thrombotic aPL manifestations (i.e. thrombocytopenia, hemolytic anemia or livedo reticularis) or a life-threatening form characterized by a rapid development of microthombosis (catastrophic APS) (Espinosa et al., 2003).

Table 2
Revised classification criteria for APS (Sydney criteria)

Clinical criteria

1. Vascular thrombosis
   One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e., unequivocal findings of appropriate imaging studies or histopathology). For histopathological confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall
2. Pregnancy morbidity
   (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10^{th} week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus
   (b) One or more premature births of a morphologically normal neonate before the 34^{th} week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions or (ii) recognized features of placental failure or
   (c) Three or more unexplained consecutive spontaneous abortions before the 10^{th} week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded

Laboratory criteria

1. LAC present in plasma, on 2 or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis
2. aCL antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titers (i.e., >40 GPL or MPL, or greater than the 99^{th} percentile), on 2 or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titers greater than the 99^{th} percentile), present on 2 or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

Note: APS is present if at least one of the clinical criteria and one of the laboratory criteria are met.

4. Primary antiphospholipid syndrome

During the early years of the description of APS, the concept of a primary APS syndrome was accepted as a preliminary step in the evolution of a full-blown lupus (Asherson, 1988). Currently, it is established that primary APS syndrome is a separate disorder and different from SLE.

Asherson (1988) described some particular characteristics in these patients, such as persistent negativity for double-stranded DNA antibodies (dsDNA), the presence of ANA at low titers (between 1:40 and 1:160), and the presence of antimitochondrial antibodies, which are also directed against phospholipids in mitochondrial membranes. Additionally, he extended the concept of primary APS to three groups of patients: (1) patients with idiopathic deep vein thrombosis (DVT), pulmonary embolism (PE), and pulmonary hypertension in the absence of any autoimmune disease, (2) patients with stroke, transient ischemic attacks (TIA) and, less commonly, other large vessel occlusions including myocardial infarction (MI) or peripheral vessel thrombosis, particularly in young patients (under the age of 45), and (3) patients with recurrent fetal losses.

Asherson et al. (1989) performed one of the first multicenter studies in patients with primary APS. Seventy patients were included, 26 (37%) were male and 44 (63%) female, giving a 2:1 female/male ratio. Mean age was 38 years (range from 21 to 59) and patients were followed for at least 5 years. None of the patients developed SLE during the follow-up. Thirty-eight (54%) patients had episodes of DVT, being accompanied by PE in 18 cases. Arterial occlusions were found in 31 (44%) patients, mainly in the form of strokes, TIA and MI. Recurrent fetal losses were present in 24 (34%) patients. Other less frequent manifestations were livedo reticularis in 14 (20%) patients and avascular necrosis in 2 (3%) patients. ANA were present in 32 (46%) patients, most of them at low titers (range from 1:10 to 1:160). Only 6 patients had ANA at high titers (from 1:320 to 1:3200). Antimitochondrial antibodies (M5 type) were present in 11 of 40 patients tested. Sixty patients were positive for LAC and aCL, 5 patients had aCL alone and 5 had only LAC. Thrombocytopenia was present in 32 (46%) patients and Coombs' positivity was present in 10, accompanied by autoimmune hemolytic anemia in 3 cases. Five out of 70 patients had relatives with SLE, rheumatoid arthritis (RA), or a clotting tendency. The authors suggested that in comparison with APS associated with SLE, patients with primary APS have a low incidence of valve lesions, livedo reticularis, chorea, fever, myalgia, and arthralgia. The presence of rheumatoid factor (RF),

cryoglobulinemia or low complement in a minority of patients might also be indicative of an immune-mediated basis in primary APS patients.

Some years later, Piette et al. (1992) proposed exclusion criteria to distinguish primary and SLE-related APS. The presence of any of these criteria excludes the diagnosis of primary APS: malar rash, discoid rash, oral or pharyngeal ulceration, frank arthritis, pleuritis in the absence of PE or left-sided heart failure, pericarditis in the absence of MI or uremia, persistent proteinuria greater than 0.5 g per day, due to biopsy-proven immune complex-related glomerulonephritis, lymphopenia (less than 1000 cells), anti-dsDNA, antiextractable nuclear antibodies (anti-ENA), ANA of more than 1:320, and drug treatment. The authors proposed that a follow-up longer than 5 years after the first clinical manifestation is necessary to rule out the subsequent emergence of SLE.

Vianna et al. (1994) analyzed the differences between primary APS and SLE-related APS. Fifty-six patients had APS plus SLE and 58 had primary APS. There were no significant differences between the two groups with the exceptions of autoimmune hemolytic anemia ($p<0.05$), cardiac valve disease ($p<0.005$), neutropenia ($p<0.01$), and low C4 levels ($p<0.001$), all of which occurred more frequently in patients with SLE-related APS.

Soltesz et al. (2003) studied a large cohort of Hungarian APS patients. Primary APS was diagnosed in 218 patients and SLE-associated APS in 288 subjects. There were significantly more men among the primary (39/218) compared with the SLE-related APS patients (27/288). Cerebrovascular thrombosis was significantly more frequent in SLE-related APS (128/288) than among primary APS patients (77/218). However, there was no difference between the two groups in the occurrence of venous thrombosis, coronary, carotid and peripheral arterial thrombosis, and fetal loss. The frequency of LAC and IgG and IgM aCL was similar in the two groups.

Primary APS is rare in children and little information exists on its potential evolution into SLE. Gattorno et al. (2003) reported one of the few series in children. The authors described 14 patients (9 boys and 5 girls) who presented clinical manifestations of APS between 3 and 13 years of age (median, 9 years) and were followed for 2–16 years (median, 6 years). Six patients presented with DVT, 5 with stroke, 2 with peripheral artery occlusion, 1 with Budd–Chiari syndrome and 1 with MI. During follow-up, 4 patients had one or more recurrences of vascular thrombosis. At the last observation, 10 patients could still be classified as having primary APS, 2 had developed SLE (both patients developed anti-dsDNA), one lupus-like syndrome and one Hodgkin lymphoma 4 years after the onset of primary APS. The authors suggested that some children who present with features of primary APS may progress to SLE or lupus-like syndrome.

However, primary APS rarely progresses to SLE. Only 8% of 128 patients, who were followed up for about 9 years, developed lupus, and a positive Coombs' test was a clinically significant predictor of progression. Gómez-Puerta et al. (2005) described 16 cases (11 with SLE and 5 with lupus-like disease) who developed clinical and/or serologic features of a "new" autoimmune disease after long-term follow-up and one patient who developed features of myasthenia gravis. Several studies have suggested that some patients with primary APS may go on to develop characteristics of SLE. To date, there are about 30 reported cases of patients whose primary APS evolved into SLE or lupus-like disease (Asherson et al., 1991; Mujic et al., 1995; Derksen et al., 1996; Seisedos et al., 1997; Blanco et al., 1999; Carbone et al., 1999; Queiro et al., 2002; Gattorno et al., 2003). The percentage of progression to SLE or lupus-like disease (21.4%) observed in pediatric patients (Gattorno et al., 2003) followed for a median of 6 years is almost double that found in the adult patients with primary APS.

Despite the heterogeneity in the clinical expression of APS, some clinical features can be grouped in different clusters. Krause et al. (2007) analyzed 246 patients with APS and, after clinical stratification and statistical analysis (by factor analysis), found different clusters for APS. The first group of patients is characterized by cardiac valves abnormalities, livedo reticularis, and neurologic manifestations (epilepsy and migraine). The second group presents the association between arthritis, thrombocytopenia, and leukopenia. The third group

presents the association between recurrent fetal loss and intrauterine growth restriction and the fourth group constitutes the inverse correlation between arterial and venous thrombosis. The authors suggested that once any of these features or lesions is recognized in a specific APS patient, special attention should be paid to for the future emergence of the other cluster manifestations.

5. Antiphospholipid syndrome associated with other diseases

APS was first recognized in patients with SLE and later was found, in a lower frequency, in patients with other autoimmune diseases. Additionally, aPL levels (either aCL or LAC) are occasionally elevated in normal individuals or can be present in a series of chronic conditions, such as infectious diseases or malignancies, or can be induced by drugs.

In a study of 552 randomly selected healthy blood donors, IgG aCL were present in up to 9.4% in a first test and were persistent in approximately 1.4% (Vila et al., 1994). Increased levels of IgG or IgM aCL have been observed in 12–52% of the elderly. The prevalence of the LAC has ranged from 1.7% of patients with suspected venous thromboembolism who did not have the disease to 8% in healthy blood donors (Shi et al., 1990). aPL also occur with increased frequency (10–15%) in women with more than three spontaneous recurrent abortions (Melk et al., 1995).

5.1. Antiphospholipid syndrome associated with autoimmune diseases

Both LAC and aCL have also been found in patients with a variety of autoimmune and rheumatic diseases (McNeil et al., 1991; Sebastiani et al., 1999; Cervera and Asherson, 2003), including hemolytic anemia, idiopathic thrombocytopenic purpura (ITP) (up to 30%) (Bidot et al., 2006), juvenile arthritis (28–46%) (Avcin et al., 2002), RA (7–50%) (Olech and Merrill, 2006), psoriatic arthritis (28%) (Buchanan et al., 1989), systemic sclerosis (25%), especially with severe disease (Picillo et al., 1995), Behçet syndrome (7–20%) (Tokay et al., 2001), primary Sjögren's syndrome (25–42%) (Fauchais et al., 2004; Ramos-Casals et al., 2006), mixed connective tissue disease (22%) (Sherer et al., 2000), polymyositis and dermatomyositis (Chakravarty et al., 1995), polymyalgia rheumatica (20%) (Chakravarty et al., 1995), chronic discoid lupus erythematosus (Ruffatti et al., 1995), eosinophilia myalgia and toxic oil syndromes (Carreira et al., 1997), vasculitis (Rees et al., 2006), and autoimmune thyroid disease (43%) (Nabriski et al., 2000), among others.

5.2. Antiphospholipid syndrome associated with infections

Since 1983, many infections have been found to be associated with aPL positivity, although the pathogenic role of these antibodies was not usually obvious except in a few isolated cases. However, there have been various reports that many infections may not only trigger the production of these antibodies but also appear to be accompanied by clinical manifestations of APS itself (Uthman and Gharavi, 2002; Ramos-Casals et al., 2004; Galrao et al., 2007). This has been seen particularly in patients with catastrophic APS (Rojas-Rodriguez et al., 2000). Some authors have proposed that infections may be a trigger for the induction of pathogenic aPL in certain predisposed subjects. The β_2GPI induced by infections may bind to "self" aPL, thus forming an immunogenic complex against which aPL are then produced. What constitutes this predisposition is unknown at this time, but clearly genetic factors might have a significant role. The antibodies produced by infectious triggers are, therefore, heterogeneous in their dependency on β_2GPI, and a minority may resemble the "autoimmune" type (Asherson and Cervera, 2003).

Viruses and other microbial agents may induce autoimmune disease by several differing mechanisms. The mechanism which concerns the production of aPL, and indeed the APS, is known as molecular mimicry. A hexapeptide, TLRVYK,

recognized specifically by a pathogenic anti-β_2GPI monoclonal antibody, was recently identified (Blank et al., 2002). These authors evaluated the pathogenic potential of microbial pathogens carrying sequences related to this hexapeptide by infusing intravenously into naïve mice IgG specific to the peptide. High titers of antipeptide anti-β_2GPI antibodies were seen in mice immunized with *Haemophilus influenzae*, *Neisseria gonorrhoea*, and tetanus toxoid. Significant thrombocytopenia, prolonged activated partial thromboplastin times, and increased fetal loss were seen. Thus, it is apparent that experimental APS can be induced by immunization with certain microbial pathogens which share epitope homology with the β_2GPI molecule (Blank et al., 2002).

Our group described the clinical and serological characteristics of 100 patients with APS related to infections (Cervera et al., 2004). Fifty-nine per cent were female and 41% male. Their mean (SD) age was 32 (18) years (range 1–78). There were 24 young patients (under 18 years), who were affected mainly by skin and respiratory infections. Sixty-eight patients had primary APS, 27 had SLE, 2 had lupus-like disease, 2 had inflammatory bowel disease (one Crohn's disease and one ulcerative colitis), and one had RA. In 40 of the 100 cases, the thrombotic events appeared in the form of catastrophic APS. The main clinical manifestations of APS included pulmonary involvement (39%), skin involvement (36%), and renal involvement (35%; 9 with renal thrombotic microangiopathy). The main associated infections and agents included skin infection (18%), human immunodeficiency virus (17%), pneumonia (14%), hepatitis C virus (13%), urinary tract infection (10%), upper respiratory infections (9%), sepsis (6%), and gastrointestinal infections (6%), among others.

It is also well known that infections are common triggers of catastrophic APS. The "CAPS Registry" shows that at least 60% of patients appear to have developed catastrophic APS following an identifiable trigger factor, with infections dominating the list. These include non-specific viral infections, pneumonia, infected leg ulcers, upper respiratory, urinary, gastrointestinal, and cutaneous infections, as well as specific infections such as typhoid fever, malaria, and dengue fever, among others (Rojas-Rodriguez et al., 2000).

5.3. Antiphospholipid syndrome associated with drugs

Several drugs have been implicated as potential inducers of APS, including phenothiazines (chlorpromazine), phenytoin, hydralazine, procainamide, quinidine, quinine, dilantin, ethosuximide, alfa interferon, amoxicillin, chlorothiazide, oral contraceptives, and propranolol (Merrill et al., 1997; Triplett, 1998).

Tumor necrosis factor alpha (TNF-α) inhibitors (adalimumab, etanercept, infliximab) have proven to be highly effective in the treatment of RA; they reduce disease activity and delay radiographic progression, with quite a good safety profile. Side-effects of anti-TNF-α treatment include an increased risk for infection and induction of autoantibodies such as ANA, anti-dsDNA, and aCL. One possible explanation for the induction of aCL positivity in patients treated with anti-TNF-α is that downregulation of TNF-α leads to upregulation of IL-10, which in turn activates autoreative B cells and thus induces autoantibody production (Atzeni et al., 2005).

Ferraccioli et al. (2002) studied the induction of aCL in eight RA patients treated with etanercept and followed during 85 weeks. Five patients presented increase of IgG aCL levels, while anti-DNA became positive in 3/8 patients. The authors have showed that the appearance of these autoantibodies correlated with bacterial urinary infection or upper respiratory tract infections, and that antibiotic treatment restored normal aCL antibody levels.

Bobbio-Pallavicini et al. (2004) studied 39 RA patients treated with infliximab followed during 78 weeks and found a significant increase in aCL titers, starting at 30 weeks for IgM antibodies and at 78 weeks for IgG antibodies. However, in most cases, the levels did not exceed normal limits, even after 78 weeks, and none of the patients exhibited any clinical features related to APS.

5.4. Antiphospholipid syndrome associated with malignancies

Since the discovery of aCL, there have been many isolated case reports of the association of aCL with vascular events in patients with a variety of malignant conditions, including solid tumors, and lymphoproliferative and hematological malignancies (Gómez-Puerta et al., 2006; Miesbach et al., 2006).

6. Catastrophic antiphospholipid syndrome

The catastrophic variant of APS is an accelerated form of this syndrome, resulting in multiorgan failure due to multiple small vessel occlusions. Since the description by Asherson (1992) more than 300 cases have been collected in the "CAPS Registry" (an international registry of patients with catastrophic APS created in 2000 by the European Forum on Antiphospholipid Antibodies, www.med.ub.es/MIMMUN/FORUM/CAPS.HTM). Patients with catastrophic APS (also currently known as Asherson syndrome (Piette et al., 2003) have in common: (a) clinical evidence of multiple organ involvement developing over a very short period of time, (b) histopathological evidence of multiple small vessel occlusions, and (c) laboratory confirmation of the presence of aPL, usually in high titers. Furthermore, approximately 60% of catastrophic episodes are preceded by a precipitating event, mainly infections, trauma, or surgical procedures, anticoagulation withdrawal, lupus flares, malignancies, or during pregnancy and the puerperium (Asherson et al., 1998, 2001, 2005; Espinosa et al., 2004; Bucciarelli et al., 2006b).

The heterogeneity of the different clinical forms of presentation led to the development of consensus criteria for the definition and classification of these patients. In September 2002, a pre-symposium workshop held during the "Tenth International Congress on aPL" in Taormina, Sicily, Italy, established preliminary criteria for the classification of the catastrophic APS (Table 3) (Asherson et al., 2003) that were later validated (Cervera et al., 2005). From the analysis of the initial 176 patients included in the "CAPS Registry," 89 (51%) of the previously included patients with catastrophic APS were classified as having "definite" and 70 (40%) as "probable" catastrophic APS. The sensitivity of the criteria was 90.3% and the specificity 99.4%. Positive and negative predictive values were 99.4% and 91.1%, respectively (Cervera et al., 2005).

Patients may develop catastrophic APS de novo, without any previous history of thrombosis, either associated with a primary APS or SLE. However, it has been shown that previous DVT, fetal loss, or thrombocytopenia are the most frequently encountered preexisting aPL-associated manifestations.

Table 3
Preliminary criteria for the classification of catastrophic APS

Evidence of involvement of three or more organs, systems and/or tissues[a]
Development of manifestations simultaneously or in less than a week
Confirmation by histopathology of small vessel occlusion in at least one organ or tissue[b]
Laboratory confirmation of the presence of antiphospholipid antibodies (LAC and/or aCL)[c]

Definite catastrophic APS:
- All 4 criteria

Probable catastrophic APS:
- All 4 criteria, except for involvement of only 2 organs, systems and/or tissues
- All 4 criteria, except for the absence of laboratory confirmation at least 6 weeks apart due to the early death of a patient never previously tested for aPL prior to the catastrophic APS event
- 1, 2 and 4
- 1, 3 and 4 and the development of a third event in more than a week but less than a month, despite anticoagulation

[a] Usually, clinical evidence of vessel occlusions, confirmed by imaging techniques when appropriate. Renal involvement is defined by a 50% rise in serum creatinine, severe systemic hypertension (>180/100 mm Hg) and/or proteinuria (>500 mg/24 h).

[b] For histopathological confirmation, significant evidence of thrombosis must be present, although vasculitis may coexist occasionally.

[c] If the patient had not been previously diagnosed as having APS, laboratory confirmation requires that aPL must be detected on 2 or more occasions at least 6 weeks apart (not necessarily at the time of the event), according to the proposed preliminary criteria for the classification of definite APS (9).

Catastrophic APS is a potentially life-threatening condition with high mortality, which requires enhanced clinical awareness. An early diagnosis and identification of potential triggering factors is essential. Once the diagnosis of catastrophic APS is confirmed, aggressive treatment is mandatory (Bucciarelli et al., 2006a, b).

However, once patients with catastrophic APS have recovered, patients usually have a stable course with continued anticoagulation. Erkan et al. (2003) documented that 66% of patients with catastrophic APS who have survived the initial event had remained symptom-free for an average follow-up of 62.7 months. Twenty-six per cent of the survivors, however, developed further APS-related events but there were no instances of further catastrophic events. Only few patients have suffered "relapsing" catastrophic APS (Bucciarelli et al., 2007). In these, clear precipitating factors were evident (e.g. recurrent infections and trauma).

7. Microangiopathic antiphospholipid syndrome

The occurrence of small vessel occlusions (thrombotic microangiopathy) in association with aPL affecting, for example, the retinal vessels, the nail fold, the skin, or major intra-abdominal organs, such as the kidney, the liver, or the bowel, is well documented. These occlusions have been described in the simple or classic APS, although these small vessel occlusions do not in any way dominate the clinical picture in the above conditions. With the description and definition of the catastrophic APS, there has been renewed interest in the thrombotic microangiopathies and their association with aPL. Although large vessel occlusions do occur in catastrophic APS, they do not dominate the clinical picture and their frequency is completely different from that encountered in the APS itself.

The term thrombotic microangiopathic hemolytic anemia (TMHA) was originally introduced by Symmers (1952) to describe a clinical state with localized or diffuse microvascular thrombosis in association with hemolytic anemia and fragmented red cells referred to as schistocytes. TMHA encompasses a spectrum of disorders including thrombotic thrombocytopenic purpura (TTP), hemolytic–uremic syndrome (HUS), malignant hypertension, postpartum renal failure, preeclampsia, and catastrophic APS. With the advent of refined testing for aPL, many cases of TMHA were published with this association (Espinosa et al., 2004) and Asherson et al. (2007) recently proposed the term microangiopathic APS to better define this subset of patients.

8. Seronegative antiphospholipid syndrome

A small group of patients have been identified who are "seronegative" at the time of the thrombotic event and, indeed, there also exists a group who are presistently seronegative and this led to the proposal of a subset termed seronegative APS (Miret et al., 1997).

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CHAPTER 2

Epidemiology of the Antiphospholipid Syndrome

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1. Classification criteria for the antiphospholipid syndrome

The antiphospholipid syndrome (APS) is a systemic autoimmune disorder defined by the presence of elevated titers of antiphospholipid antibodies (aPL), thromboembolic events, and pregnancy morbidity. In 1983, Hughes first described the association between thrombosis and aPL in patients with systemic lupus erythematosus (SLE) (Hughes, 1983). Classification criteria for APS were developed by consensus at the Sapporo antiphospholipid meeting (Wilson et al., 1999). The 1999 International Consensus Statement on Preliminary Criteria for the Classification of the Antiphospholipid Syndrome were the first to recognize that vasculopathy (and not just thrombosis) were part of APS and broadened the pregnancy morbidity criterion to include severe preeclampsia (Wilson et al., 1999; Petri, 2006). These criteria defined patients with "primary" APS as those with both clinical and laboratory criteria for the diagnosis in the absence of underlying autoimmune disease, and patients with "secondary" APS as those with autoimmune disorders and thrombosis that were found to have the presence of aPL (Wilson et al., 1999).

The "primary" versus "secondary" APS distinction was eliminated in the 2006 Sydney International Consensus Statement on an Update of the Classification Criteria for Definite Antiphospholipid Syndrome (Miyakis et al., 2006). Instead, the 2006 criteria defined two subgroups of APS patients, namely those with and those without the presence of coexisting inherited and acquired risk factors for arterial or venous thrombosis (Miyakis et al., 2006). These non-aPL thrombosis risk factors include, but are not limited to, age (>55 in men, and >65 in women), the presence of any of the established risk factors for cardiovascular disease, inherited thrombophilias, oral contraceptives, nephrotic syndrome, malignancy, immobilization, and surgery (Miyakis et al., 2006).

The 2006 revised clinical classification criteria for APS have essentially remained the same as the 1999 clinical Sapporo criteria, while the laboratory criteria have changed considerably. According to both the original and revised criteria, a patient with definite APS must have persistent high-titer aPL associated with one or more clinical episodes of arterial, venous, or small vessel thrombosis, occurring within any tissue or organ (Wilson et al., 1999; Miyakis et al., 2006). The revised 2006 clinical criteria have extended the thrombosis criterion for APS to include other cardiovascular diseases such as transient cerebral ischemia, stroke, intracardiac thrombi, and biopsy-proven myocardial microthrombosis (Miyakis et al., 2006).

Laboratory requirements for both the Sapporo and the revised classification criteria for diagnosis

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of definite APS include anticardiolipin (aCL) IgG or IgM or lupus anticoagulant (LAC). However, the original criteria state that aPL must be present in medium or high titers, while the revised criteria more specifically define the titers as greater than 40 IgG phospholipid units (GPL) or IgM phospholipid units (MPL) or titers in the 99th percentile (Wilson et al., 1999; Miyakis et al., 2006). In contrast to the 1999 international criteria, the revised 2006 criteria include the presence of antibodies to IgG and/or IgM anti-β_2 glycoprotein I (anti-β_2GPI) as part of the laboratory criteria for the diagnosis of APS (Miyakis et al., 2006). In addition, all aPL are required to be positive on more than one occasion at least 12 weeks apart in the updated laboratory criteria, as opposed to 6 weeks apart in the Sapporo criteria (Wilson et al., 1999; Miyakis et al., 2006). The rationale behind the committee's increase of the interval to 12 weeks was to alleviate concerns that the transient presence of epiphenomenal aPL could risk misclassification (Male et al., 2005), and to provide greater reassurance that the aPL detected are associated with a predisposition to APS (Miyakis et al., 2006). Finally, the revised 2006 criteria classify patients into four groups of aPL, and investigators are strongly advised to use these distinctions in their clinical studies (Miyakis et al., 2006). The aPL subcategories are the following: I, more than one laboratory criterion present (any combination); IIa, LA present alone; IIb, aCL antibody present alone; IIc, anti-β_2GPI antibody present alone (Miyakis et al., 2006).

2. Probable antiphospholipid syndrome

The members of the 2006 international panel also discussed clinical and laboratory features not included in the revised classification criteria for APS. A classification of "probable APS" has been given to patients positive for aPL that have clinical features such as livedo reticularis, valvular heart disease, nephropathy, thrombocytopenia, or neurological manifestations to suggest APS, but lack the clinical criteria of vascular thrombosis or pregnancy loss to be defined as "definite" APS (Miyakis et al., 2006; Asherson, 2006). Members proposed that "probable APS" could also be used to classify infrequent cases of patients that fulfill the clinical criteria for the diagnosis, but test positive for non-criteria aPL, such as IgA aCL, IgA anti-β_2, antiphosphatidylserine antibodies (aPS), antiphosphatidylethanolamine antibodies (aPE), antibodies against prothrombin alone (aPT-A), and antibodies to the phosphatidylserine–prothrombin complex (aPS/PT) (Asherson, 2006).

3. Classification criteria for catastrophic antiphospholipid syndrome

Catastrophic antiphospholipid syndrome (CAPS), also referred to as Asherson's syndrome, is another subset of APS that is characterized by acute thrombotic microangiopathy with subsequent multiorgan failure and a high mortality rate (Asherson et al., 1998). Criteria for diagnosis of CAPS were proposed by the 2002 International Taormina Consensus Statement of Classification and Treatment of CAPS (Asherson, 2003). For diagnosis of definite CAPS, there should be involvement of three or more organ systems and/or tissues, the clinical manifestations should develop simultaneously or in less than one week, and there should be confirmation by histopathology for small vessel occlusion and by laboratory for the presence of aPL (Asherson, 2003). Another subset of APS that has been proposed is microangiopathic APS (MAPS), which may include CAPS patients that have only small vessel involvement (Asherson, 2006).

4. Validation of antiphospholipid syndrome classification criteria

There are very few studies validating the 1999 Sapporo criteria for APS and the subsequent 2006 Sydney update of the classification criteria (Lockshin et al., 2000; Pourrat et al., 2006; Kaul et al., 2007). Lockshin and colleagues tested the Sapporo criteria for classification of APS in a prospective cross-sectional study of 243 patients

with clinical diagnoses of primary or secondary APS, SLE without clinical APS, and lupus-like disease without clinical APS (Lockshin et al., 2000). Sensitivity, specificity, positive predictive value, and negative predictive value were 0.71, 0.98, 0.95, and 0.88, respectively (Lockshin et al., 2000). The authors concluded that the Sapporo criteria were usable for clinical studies as they were sensitive and specific for the classification of primary and secondary APS, and compared favorably with the American College of Rheumatology criteria for SLE (Lockshin et al., 2000). Despite the high sensitivity and specificity of the Sapporo criteria, concern has been expressed that these criteria would perform poorly in populations with high frequencies of aPL, such as hospitalized patients with thromboembolic disease or the elderly (Miyakis et al., 2006). Miyakis and colleagues noted in their 2006 update of classification criteria for APS that the association of aging and of traditional risk factors for cardiovascular disease with thrombosis may cause classification bias (Cooper et al., 2000; Miyakis et al., 2006).

More recently, Kaul and colleagues assessed the 2006 revised APS classification criteria in a study of 200 aPL-positive patients (Kaul et al., 2007). Patients were analyzed for fulfillment of the 1999 Sapporo and 2006 revised APS classification criteria. Of the 144 aPL-positive patients who met the laboratory requirement of the 1999 Sapporo criteria, only 59% met the laboratory requirement of the 2006 revised classification criteria, resulting in a more selective aPL-positive patient population (Kaul et al., 2007). Four patients (10%) that did not meet the 1999 Sapporo criteria for classification of APS qualified as APS in the revised 2006 criteria because of the presence of anti-β_2GPI alone (Kaul et al., 2007).

Pourrat and colleagues had similar findings when they applied the updated 2006 classification criteria for APS in a retrospective study of 107 aPL-positive women seen at an obstetric medicine clinic (Pourrat et al., 2006). The authors found six new definite cases of APS when including the updated laboratory criteria of anti-β_2GPI positivity (Pourrat et al., 2006).

In addition to validating the updated criteria for APS, Kaul and colleagues also examined non-criteria aPL features and non-aPL thrombosis risk factors at the time of vascular events based on the 2006 revised consensus statement definitions. Investigators found that non-criteria aPL clinical features occurred more frequently in patients with APS with vascular events, the most common being livedo reticularis (Kaul et al., 2007). The most common coexisting non-aPL risk factor in patients with a vascular event with or without pregnancy morbidity was smoking (16% of patients), followed closely by oral contraceptive pills or hormone replacement therapy (15% of patients) (Kaul et al., 2007). About half of the patients in this cohort with vascular events had at least one identifiable non-aPL thrombosis risk factor at the time of their vascular events (Kaul et al., 2007). This finding is similar to another study by Giron-Gonzalez and colleagues, in which at the time of the first thrombotic event, 50% of patients with APS had coincident risk factors for thrombosis (Giron-Gonzalez et al., 2004). It is believed that the revised classification criteria will have positive implications in APS research by providing a more selective and risk-stratified framework for evaluating aPL-positive patients (Kaul et al., 2007).

The results of Kaul and colleagues' validation of the revised 2006 APS criteria were further evaluated in a commentary by Cabral and Cabiedes (2007). The authors reported that of the 82 asymptomatic patients analyzed, 39 met at least one serological criterion for APS (Cabral and Cabiedes, 2007). Assuming that the remaining 43 patients were asymptomatic and aPL-negative, the sensitivity and specificity of aCL alone for APS is 0.28 and 0.67, 0.09 and 0.98 for anti-β_2GPI alone, 0.28 and 0.59 for more than one aPL and 0.52 and 0.52 for all serological criteria, respectively (Cabral and Cabiedes, 2007). They noted that these findings support previous data showing that aCL have high sensitivity and low specificity for APS, while anti-β_2GPI have higher specificity and lower sensitivity than aCL for APS (Bertolaccini and Khamashta, 2006). The authors concluded that a large multinational study is needed to objectively and prospectively validate the revised criteria (Cabral and Cabiedes, 2007).

5. Predictors of antiphospholipid syndrome

It is thought that thrombosis is more likely to occur with the existence of two or more risk factors that can affect the venous or arterial beds, including stasis, vascular injury, and the use of medications such as oral contraceptives (Levine et al., 2002). In patients with aPL the risk of thrombosis is increased with the addition of prothrombotic heritable risk factors, including factor V Leiden and prothrombin mutation G20210A (Brouwer et al., 2004). Moreover, patients with a history of thrombosis or fetal loss secondary to APS are more likely to also have genetic risk factors than asymptomatic individuals with aPL (Forasterio et al., 2001). Other risk factors for arterial complications, such as migraines, atherosclerotic disease, or hyperlipidemia might be increased in SLE patients positive for aPL, and could further increase the risk for stroke or myocardial infarction (MI) (Sammaritano, 2007).

The importance of a coexisting risk factor serving as a "second hit" to explain the occurrence of thrombosis has been supported in several reports (Krnic-Barrie et al., 1997; Giron-Gonzalez et al., 2004). In a prospective study of 404 individuals, Giron-Gonzalez and colleagues compared the clinical and analytical findings from patients with APS ($n = 226$) and asymptomatic carriers of aPL (Giron-Gonzalez et al., 2004). In contrast to asymptomatic carriers of aPL, a significant proportion of patients with APS were found to have a coexisting risk factor for vascular disease at the time of their first thrombotic event (50% vs. 27.5%, respectively, $p < 0.001$) (Giron-Gonzalez et al., 2004). Furthermore, risk factors were different in the cases of arterial compared with venous thrombosis. Hypercholesterolemia (27.4%) or arterial hypertension (24.2%) were significantly associated with arterial thrombosis (31% of APS patients), while immobilization (14.2%) and previous surgery (17.9%) were more frequently detected in venous thrombosis (46.9% of APS patients) (Giron-Gonzalez et al., 2004). Similarly, in a retrospective review of 61 patients with APS, hypertension ($p = 0.01$) and hyperlipidemia ($p = 0.03$) were significantly associated with arterial thrombotic events, but only white race was associated with recurrent arterial events (Krnic-Barrie et al., 1997). In conclusion, it is likely that secondary risk factors may help determine which patients develop clinical manifestations of APS (Hanley, 2003). Thus, eliminating or reducing the effect of these factors is especially important, since the presence of aPL may require a "second hit" for thrombosis to occur (Levine et al., 2002).

6. Prevalence of antiphospholipid antibodies in arterial thrombosis

Arterial thrombosis occurs less frequently than venous thrombosis in the APS (Vianna et al., 1994; Cervera et al., 2002) and is most commonly characterized by ischemia or infarction (Levine et al., 2002). The cumulative retrospective literature indicates that approximately 30–40% of patients with aPL have a history of thrombosis and that 30% of the events are arterial (Love and Santoro, 1990; Finazzi, 2001). The most common arterial site affected is the cerebral circulation (Giron-Gonzalez et al., 2004), with approximately half of arterial occlusions attributed to strokes and transient ischemic attacks, while another 23% are attributed to coronary occlusions (Asherson et al., 1989). Investigators of the Euro-Phospholipid Group found the prevalence of arterial complications, such as MI and arterial thrombosis in the lower extremities, was higher in men than in women (16% vs. 3%, $p < 0.001$ and 11% vs. 3%, $p < 0.001$, respectively) (Cervera et al., 2002). In addition, patients with older onset APS were also more frequently male and experienced more arterial manifestations of stroke and angina pectoris than the rest of the cohort (30% vs. 18%, $p < 0.005$ for stroke and 9% versus 2%, $p < 0.001$ for angina pectoris, respectively) (Cervera et al., 2002).

The association of aPL with recurrent stroke has been demonstrated in prospective studies (Levine et al., 1990, 1992; Rosove and Brewer, 1992). Patients with cerebral ischemia and APS are younger than the general stroke population (Brey et al., 1990). In particular, the strongest association of aPL with stroke is seen in patients under 50 years of age, with a prevalence of aPL reported

to be from 4 to 46% in this population (Hart et al., 1984; Brey et al., 1990; Nencini et al., 1992; Petri, 2000). Nencini and colleagues prospectively tested aPL in 55 young adults (age 15–44 years) consecutively examined for ischemic stroke or transient ischemic attack, and reported that patients with IgG or IgM aCL >20 units had a higher probability of cerebral ischemic or systemic thrombotic events during follow-up than patients without (Nencini et al., 1992). In contrast, evidence has shown that low positive aCL titers are not an independent risk factor for stroke (Sletnes et al., 1992; Silver et al., 1996). Nencini and colleagues' study of LA and aCL in young adults with cerebral ischemia reported that 18% of their patients had aPL, all with stroke (Nencini et al., 1992). This prevalence is higher than the 8–25% of aPL reported in prospective case–control studies of stroke patients of all ages (Hess et al., 1991; APASS, 1997; Zielinska et al., 1999).

The Antiphospholipid Antibodies and Stroke Study Group (APASS) previously studied 248 unselected stroke patients and demonstrated aCL positivity in 9.7% of patients and 4.3% of controls, with an odds ratio of aCL positivity for stroke of 2.3 (APASS, 1993). The same investigators subsequently performed a prospective follow-up study of individuals with a first ischemic stroke and IgG aCL positivity, and found the risk of recurrent stroke to be 11.1% over a mean of approximately 3 years (APASS, 1997). Other studies have found similar cumulative stroke recurrence rates in patients with aPL, such as Lai and colleagues with 13% after 2 years, the Oxfordshire Community Stroke Project with 13.2% at 1 year and 19.9% at 2 years, and Levine and colleagues with 13% at 1 year (Levine et al., 1990; Burn et al., 1994; Lai et al., 1994).

Finally, a meta-analysis of 11 case–control, cross-sectional, and ambispective studies was performed to evaluate 22 associations between IgG and/or IgM aCL and arterial and/or venous thrombosis in 1883 cases and 2469 controls (Galli et al., 2003b). Five studies analyzed eight associations with the first cerebral stroke, and a significant 95% confidence interval was found in six of the associations (the odds ratio ranged from 1.35 to 6.67) (Galli et al., 2003b). Three studies analyzed six associations of aCL with MI and found that all but one study showed a significant 95% confidence interval, irrespective of the recurrence of event or the antibody isotype(s), with the odds ratio ranging from 1.21 to 18 (Galli et al., 2003b). In a separate analysis of the different types of thrombosis, the investigators found that aCL were associated with cerebral stroke and MI but not with DVT, while lupus anticoagulants were strong risk factors for both arterial and venous thrombosis (Galli et al., 2003b).

The association between aPL and MI has been suggested in several other studies (Vaarala et al., 1995; Wu et al., 1997; Glueck et al., 1999). Glueck and colleagues demonstrated that aCL are independent risk factors for atherosclerotic vascular disease in a cross-sectional study of 864 patients referred for diagnosis and treatment of hyperlipidemia (Glueck et al., 1999). Atherosclerotic events were more common in men than women, and for patients having a MI or coronary artery bypass surgery and/or angioplasty, aCL IgM was a significant independent positive predictor of events (odds ratio of 2.38 in patients with top decile aCL IgM) (Glueck et al., 1999). Moreover, aCL IgM was a positive significant risk factor for MI in patients 55 years or younger, with an odds ratio of 2.79 in subjects with top decile aCL IgM (Glueck et al., 1999).

In a prospective cohort of healthy middle-aged men participating in the Helinski Heart Study (a 5-year coronary primary prevention trial with gemfibrozil), Vaarala and colleagues studied whether the presence of aPL carries a risk for MI (Vaarala et al., 1995). In a nested case–control design, 133 patients with MI or cardiac death were compared with 133 controls matched for treatment (gemfibrozil vs. placebo) and geographical region (Vaarala et al., 1995). By logistic regression analysis, aCL IgG was higher in patients than controls ($p < 0.005$), and patients with aCL IgG in the highest quartile had a relative risk for MI of 2.0 (95% confidence interval 1.1 to 3.5) compared with the rest of the cohort (Vaarala et al., 1995). This increased risk of MI associated with aCL IgG was independent of any confounding factors, such as age, smoking, systolic blood pressure, low-density and high-density lipoprotein

(Vaarala et al., 1995). Thus, the investigators concluded that the presence of a high aCL antibody level is an independent risk factor for MI or cardiac death (Vaarala et al., 1995). An association between aCL and MI was found in another prospective nested case–control study by Wu et al. (1997). Investigators followed 119 healthy men for up to 20 years and found that high levels of antibodies against oxidatively modified low-density lipoproteins (oxLDL) and cardiolipin at 50 years of age was correlated positively with the incidence of MI and mortality related to MI 10–20 years later ($p<0.05$) (Wu et al., 1997). Specifically, men who acquired MI had a higher prevalence of IgG aCL, and the risk for individuals with IgG and IgA aCL at 50 years of age to develop MI within a 20-year period was 2.7 and 3.0, respectively (Wu et al., 1997). Higher levels of aCL and oxLDL were detected in those who died from acute MI compared with those who survived ($p<0.05$) (Wu et al., 1997). The authors concluded that IgA and IgG antibodies to anticardiolipin and oxLDL were strong and independent risk factors for the prediction of MI and MI-related death (Wu et al., 1997).

7. Prevalence of antiphospholipid antibodies in venous thrombosis

The most common manifestation of APS is venous thrombosis, which usually presents as DVT in the lower extremities, and occurs in 29–55% of patients with the syndrome over a follow-up period of less than 6 years (Asherson et al., 1989; Vianna et al., 1994). More than half of these patients have pulmonary emboli (Alarcon-Segovia et al., 1992; Provenzale et al., 1998). In patients with APS, Schulman and colleagues determined that the risk for recurrence of venous thrombosis doubles to at least 30% without thrombotic therapy (Schulman et al., 1998).

The clinical manifestations of venous thromboembolism (VTE) are similar in patients with or without aPL (Baker and Bick, 2008). However, thrombotic episodes associated with the APS may commonly occur in vascular beds that are infrequently affected by other prothrombotic states or unusual for the general population, such as the superior vena cava, subclavian vein, and abdominal and pelvic vessels (Provenzale et al., 1998). Cerebral venous thrombosis caused by aPL differs from onset of cerebral venous thrombosis related to other causes, as it usually presents at a younger age and is more extensive (Baker and Bick, 2008). A series of 40 patients with cerebral venous thrombosis reported three cases had aPL, and two of these three also had a factor V Leiden mutation (Deschiens et al., 1996). This study emphasizes that testing for aPL may be positive in patients with a family history of venous thrombosis, because many patients with one thrombophilic disorder also have another (Brouwer et al., 2004).

Prospective studies in the general population have demonstrated that aPL are predictive of both a first DVT (Ginsberg et al., 1992) and recurrent thromboembolism and death (APASS, 1997; Schulman et al., 1998). Using a cohort of healthy adult men drawn from the Physicians' Health Study, Ginsberg and colleagues performed a prospective case–control study to determine whether the presence of aCL increased the risk for venous thrombosis (Ginsberg et al., 1992). The authors found that aCL titers were higher in physicians with DVT and pulmonary embolism (PE) ($p = 0.01$) (Ginsberg et al., 1992). In addition, physicians with aCL titers greater than the 95th percentile had a relative risk for developing DVT or PE of 5.3 (95% confidence interval 1.55 to 18.3, $p = 0.01$) (Ginsberg et al., 1992). Therefore, it was concluded that the presence of aCL was a risk factor for DVT or PE in healthy adult men, independent of age and smoking status (Ginsberg et al., 1992).

The prevalence of aPL in patients with venous thrombosis has been determined in a number of cross-sectional studies (Bick et al., 1992; Mateo et al., 1997; Eschwege et al., 1998; Salomon et al., 1999; Zanon et al., 1999). Frequencies of aPL in venous thrombosis have been reported to be from 5.2% to 30% for any aPL, 0.6–16% for antibodies to LAC, and 4–24% for aCL (Petri, 2000). Supporting these prevalence data is a series of 100 consecutive patients with idiopathic DVT or PE in which 24% were found to have aPL (Bick et al., 1992).

As the revised 2006 classification criteria have updated the laboratory criteria for the diagnosis of APS to include the presence of antibodies to IgG and/or IgM anti-β_2 glycoprotein I (anti-β_2GPI) (Miyakis et al., 2006), there has been growing interest to study the clinical significance of this aPL. The association of increased anti-β_2GPI in patients with VTE has predominantly been obtained from analysis of retrospective data (Bas de Laat et al., 2005; Zoghlami-Rintelen et al., 2005; Danowski et al., 2006). A cohort of 87 consecutive patients persistently positive for LA, 55 with and 32 without a history of thrombosis, was retrospectively investigated to determine whether anti-β_2GPI or aCL were associated with an increased risk of thrombosis (Zoghlami-Rintelen et al., 2005). In respect to venous events ($n = 47$), 37 patients (79%) had a history of DVT, 8 (17%) PE, and 2 (4%) DVT plus PE (Zoghlami-Rintelen et al., 2005). Elevated anti-β_2GPI IgG significantly increased the odds for VTE by about fivefold (odds ratio 5.2, 95% confidence interval 1.5–18), and had a stronger risk association for VTE than elevated aCL IgG (Zoghlami-Rintelen et al., 2005). These data correspond with a study by Wahl and colleagues in which a higher recurrence rate of VTE was found in non-SLE patients with anti-β_2GPI (Wahl et al., 1998a).

In a cross-sectional study of 418 consecutive patients with SLE or APS, the prevalence of anti-β_2GPI was 44.5%, compared with 55.5% for aCL, and 31.1% for LA (Danowski et al., 2006). Specifically, 15.7% of patients with any anti-β_2GPI had venous thrombosis, while 12.4% of patients with any anti-β_2GPI had arterial thrombosis (Danowski et al., 2006). This series was the first to apply the 12-week persistent positivity rule from the Sydney revision of the Sapporo APS classification criteria (Miyakis et al., 2006). Persistent positivity increased the association of IgG anti-β_2GPI with venous thrombosis and IgM anti-β_2GPI with arterial thrombosis (odds ratio 6.37, 95% confidence interval 1.82–22.31, $p = 0.0125$ and odds ratio 5.63, 95% confidence interval 1.40–22.6, $p = 0.0455$, respectively) (Danowski et al., 2006).

Many other groups have also found an association between anti-β_2GPI and thrombosis, as reviewed by Galli et al. (2003a). On analysis of 5102 patients and 1973 controls, 34 of 60 (57%) associations of anti-β_2GPI and thrombosis reached significance, including 5 of 17 with arterial thrombosis, 12 of 21 with venous thrombosis, and 17 of 22 with any thrombosis (Galli et al., 2003a). Analysis in relation to the type of thrombosis revealed that anti-β_2GPI antibodies seemed more often associated with venous thrombosis, and aCL with arterial thrombosis (Galli et al., 2003a).

Wahl and colleagues assessed the risk of venous thrombosis in individuals with aPL in a meta-analysis (Wahl et al., 1998b). Excluding patients with SLE or previous thrombosis, they evaluated seven observational studies and found the overall odds ratio for LA-associated VTE was 11.1 (95% confidence interval 3.81–32.3) (Wahl et al., 1998b). Their meta-analysis of studies showed that the lupus anticoagulant had a stronger association with VTE than did aCL (Wahl et al., 1998b).

8. Prevalence of antiphospholipid antibodies in pregnancy morbidity

While pregnancy loss in the general population is most frequent during the first 9 weeks of gestation, women with aPL have a high risk of pregnancy loss from the 10th week of gestation onward (fetal period) (Lockshin et al., 1985). Both the 1999 Sapporo and 2006 Sydney International Consensus Statements have recognized these facts and defined obstetric manifestations of APS to include one or more unexplained fetal death at or beyond the 10th week of gestation, premature birth before the 34th week of gestation because of eclampsia, severe preeclampsia, or placental insufficiency, or three or more losses before the 10th week of gestation (Wilson et al., 1999; Miyakis et al., 2006). Multiple cross-sectional studies have reported an association of aCL and/or LA with recurrent fetal loss, with a frequency in a general range of 10–19% (Petri, 2006). In addition to fetal loss, other pregnancy complications in women with aPL have been observed, such as intrauterine growth retardation, HELLP syndrome (hemolytic

anemia, elevated liver enzymes and low platelet count), oligohydramnios, premature birth related to pregnancy-associated hypertension and uteroplacental insufficiency (Lima et al., 1996; Levine et al., 2002; Baker and Bick, 2008). The occurrence of subsequent arterial or venous thrombosis has been shown in patients with such obstetric complications (Carp, 2004). Due to high maternal and/or fetal mortality, contraindications of pregnancy in women with APS include uncontrolled arterial hypertension, recent (<6 months) thrombotic event, or pulmonary hypertension (Ruiz-Irastorza and Khamashta, 2007).

Preeclampsia, a complication associated with placental insufficiency, occurs in up to 50% of pregnancies in women with the APS (Branch et al., 1992). In half of the cases preterm delivery is required because of severity of the disease (Branch et al., 1992). The prevalence of aCL is 11–29% in pregnancies complicated by preeclampsia (Yasuda et al., 1995; Allen et al., 1996; Van Pampus et al., 1999). Moreover, the presence of aCL are detected more frequently in patients with early-onset severe preeclampsia before the 34th week of gestation (Branch et al., 1989). In a recent systematic review, aPL was demonstrated to be one of the most significant risk factors for preeclampsia (Duckitt and Harrington, 2005). However, as evidenced by Lee and colleagues in their lack of correlation between aCL or anti-β_2GPI and preeclampsia, there are conflicting results on whether women with preeclampsia have a higher prevalence of aPL (Lee et al., 2003b).

In a prospective, multicenter study of 1000 patients with APS, the Euro-Phospholipid Group determined that the most common fetal complications seen in their cohort were early fetal loss (35.4% of pregnancies), late fetal loss (16.9% of pregnancies), and premature birth (10.6% of live births), while the most common maternal complications were preeclampsia (9.5% of pregnant women) and eclampsia (4.4% of pregnant women) (Cervera et al., 2002). Similarly, Giron-Gonzalez and colleagues determined prospectively that, of women of fertile age with APS (non-SLE), 53.7% suffered from fetal loss, with a higher risk of spontaneous abortion linked to the existence of previous fetal losses (Giron-Gonzalez et al., 2004). Finazzi and colleagues reported that women with a history of miscarriages or vascular occlusions had a significantly higher rate of adverse pregnancy outcome (56%) than asymptomatic women (17%), in their 4-year prospective study of 360 unselected patients with aPL ($p = 0.035$) (Finazzi et al., 1996). In another prospective study of 325 patients, Lynch and co-workers found an association of aCL and antiphosphatidylserine with all pregnancy losses but no association was determined between anti-β_2GPI antibodies and all pregnancy losses (Lynch et al., 1999). Some studies have failed to find an association between aPL and pregnancy losses (Teixido et al., 1997; Simpson et al., 1998).

More recently, a meta-analysis by Opatrny and colleagues assessed the relationship between aPL and recurrent fetal loss in women without autoimmune disease (Opatrny et al., 2006). Twenty-five published case–control, cohort, and cross-sectional studies rated moderate or strong were included in the meta-analysis (Opatrny et al., 2006). Lupus anticoagulant was the most strongly associated aPL with recurrent fetal losses before 24 weeks' gestation (odds ratio 7.79, 95% confidence interval 2.30–26.45) (Opatrny et al., 2006). IgG and IgM aCL were also associated with recurrent fetal losses before 24 weeks' gestation (odds ratio 3.57, 95% confidence interval 2.26–5.65 and odds ratio 5.61, 95% confidence interval 1.26–25.03, respectively) (Opatrny et al., 2006). IgG aCL was also related to early recurrent fetal losses before 13 weeks' gestation (odds ratio 3.56, 95% confidence interval 1.48–8.59) (Opatrny et al., 2006). No significant association was found between anti-β_2GPI and early recurrent fetal losses (odds ratio 2.12, 95% confidence interval 0.69–6.53) (Opatrny et al., 2006). However, the investigators did not present data on recurrent fetal losses after 24 weeks' gestation, and noted that the wide confidence interval with the association of early miscarriage and anti-β_2GPI suggested a power problem (Opatrny et al., 2006). The authors concluded that the strength of the association between aPL and recurrent fetal loss depends on the type of aPL, and the role of testing for anti-β_2GPI remains to be established (Opatrny et al., 2006).

The presence of APS is a previously identified risk factor for poor pregnancy outcome in women with SLE (Lockshin et al., 1985; Clowse et al., 2005). Supporting this is a study by Clowse and colleagues, in which 166 pregnancies in the Hopkins Lupus Cohort were followed from the first trimester onward (Clowse et al., 2006). A diagnosis of secondary APS led to an increase in pregnancy loss by 3.1-fold ($p = 0.004$) (Clowse et al., 2006). Furthermore, the presence of aCL and/or LA without the clinical criteria for secondary APS did not increase the risk for pregnancy loss among lupus patients (Clowse et al., 2006).

9. Prevalence of non-criteria clinical manifestations in antiphospholipid syndrome

Definitions of features of APS that were not included in the updated criteria were provided in the 2006 International consensus statement for classification of definite APS (Miyakis et al., 2006). These included heart valve disease, livedo reticularis, thrombocytopenia, nephropathy, neurological manifestations, and various aPL mentioned previously in this chapter (IgA aCL and anti-β_2GPI, aPS, aPE, aPT-A, aPS/PT) (Miyakis et al., 2006). Although these features are frequently associated with APS, the 2006 consensus committee believed that their adoption as independent criteria for definite APS may decrease diagnostic specificity (Miyakis et al., 2006). However, the APS classification criteria do not specify the pathogenetic pathways, and some manifestations of APS (chorea, transverse myelitis, early pregnancy losses) may be the consequence of an inflammatory action of aPL rather than thrombotic (Petri, 2004).

As evidenced by multiple supportive prospective cohort and case–control studies in patients with and without SLE, cardiac valvular disease (vegetations and valve thickening) are frequent in APS (Petri, 2004). In a series by Zavaleta and colleagues 71% of patients with primary APS were found to have cardiac valve lesions (Zavaleta et al., 2004). However, there are inconsistencies in the data because of differences in associations with aPL, echocardiography technique, and population heterogeneity (Cervera, 2004).

Thrombocytopenia is seen in 20–40% of APS patients and is usually mild, rarely presenting with bleeding complications (Galli et al., 1996). Thrombocytopenia is seen more frequently in patients with APS and SLE than in patients with APS alone (Cervera et al., 2002). The 2006 revised classification criteria proposed a platelet count of less than 100 000 as the upper cut-off limit for thrombocytopenia in APS (Miyakis et al., 2006). However, thrombocytopenia is not included as an independent criterion for APS, as data from prospective studies have not clearly distinguished thrombocytopenia associated with persistent aPL from thrombocytopenia related to SLE or idiopathic thrombocytopenic purpura (ITP) (Miyakis et al., 2006). In fact, in a prospective study of 82 ITP patients, 37.8% were aPL-positive at diagnosis (Diz-Kucukkaya et al., 2001). After a median follow-up of 38 months, 14 ITP patients (45%) who had aPL developed clinical features of APS (Diz-Kucukkaya et al., 2001). This may suggest that aPL is a risk factor for the development of thrombosis in patients with ITP (Diz-Kucukkaya et al., 2001), or that ITP is the first symptom of APS, as may be seen in SLE (Miyakis et al., 2006).

The ''Euro-Phospholipid'' project is a multicenter, consecutive and prospective study of 1000 patients with APS (Cervera, 2008). The cohort was started in 1999 and designed in order to analyze the prevalence and characteristics of the most relevant clinical and immunological features of APS (Cervera et al., 2002). Several non-criteria manifestations that were frequently found include thrombocytopenia (29.6%), livedo reticularis (24.1%), and heart valve lesions (14.3%) (Cervera et al., 2002). Livedo reticularis is a prominent manifestation of APS, present in 11–22% of patients (Asherson et al., 1989; Alarcon-Segovia et al., 1992; Vianna et al., 1994), and as seen in the Euro-Phospholipid cohort, is even more prevalent among APS patients with SLE and in women (Cervera et al., 2002). The prevalence of other clinical features that have occasionally been seen in APS patients include arterial thrombosis in the legs (4.3%) and arms (2.7%), subclavian (1.8%) and jugular (0.8%) vein thrombosis, multi-infarct

dementia (2.5%), chorea (1.3%), and pulmonary hypertension (2.2%) (Cervera et al., 2002). Chorea has been strongly associated with the presence of aPL, and it may even precede the development of aPL (Baker and Bick, 2008). In a study of 38 patients with precapillary pulmonary hypertension, the reported frequency of aPL was high at 30% (Karmochkine et al., 1996). Finally, the prevalence of other manifestations of APS has been reported as less than 1%, such as transverse myelopathy and Addison's syndrome (Cervera et al., 2002).

10. Prevalence of antiphospholipid antibodies in the general population

It is important to ascertain the prevalence of aPL in the general population in order to be able to perform accurate case–control studies in APS (Petri, 2000). Several studies have evaluated the presence of aPL in normal controls, and the data indicate that the prevalence is 1–5% for both aCL and LA antibodies among healthy young people (Shi et al., 1990; Juby and Davis, 1998; Petri, 2000). Creagh and colleagues reported a prevalence of aCL or LA as 3% in 500 pregnant women (Creagh et al., 1991). In another large cohort of 510 healthy pregnant women studied prospectively, the prevalence of anti-β_2GPI and aCL at 15–18 weeks was determined to be 3.9% and 1.6%, respectively (Faden et al., 1997). However, the frequency of antibodies to β_2GPI in other populations of healthy normal individuals has not been determined (Bas de Laat et al., 2004a). The prevalence of aPL increases with age, especially among the elderly with chronic disease, with up to 50% of elderly people reportedly positive for aPL (Manoussakis et al., 1987; Juby and Davis, 1998; Petri, 2000).

Many control subjects have laboratory evidence for aPL and suffer no clinical consequences (Levine et al., 2002). However, it is unclear if some of these patients will eventually develop symptoms of APS (Levine et al., 2002). In most cases of normal controls, the titers of aPL are low-positive and transient (Finazzi, 2001). In the study of 510 healthy pregnant women by Faden and colleagues, anti-β_2GPI was associated with pre-eclampsia–eclampsia, but not with fetal loss, abruption placentae, and fetal growth retardation (Faden et al., 1997). Conversely, in a study by Vila and colleagues of 552 normal blood donors, the prevalence of IgG aCL was 6.5% and 9.4% for IgM aCL, and no thrombotic event occurred in donors that were positive for aCL during a 12-month follow-up (Vila et al., 1994).

11. Problems with assays and different study designs in the assessment of antiphospholipid syndrome

The results of clinical studies are greatly influenced by factors such as differences in study design and eligibility criteria, making comparisons of studies in the epidemiology of APS challenging (Galli et al., 2003b). Information on how long aPL have been present and the risk of thrombosis in patients can be provided by prospective, ambispective, cross-sectional, and case–control studies (Galli et al., 2003b). Case–control studies represent the foundation of clinical studies in APS. However, the reliability of the results are dependent on the correct ascertainment of previous thrombosis and frequency of aPL in normal controls (Petri, 2000). Both case–control and cross-sectional studies determine the strength of the association between aPL and thrombosis, though a potential limitation is that they are based on the premise that the level and type of aPL measured at or after the event are indicative of the antibody status before the event (Galli et al., 2003b). Prospective and ambispective studies overcome most of these hurdles and are good for evaluating the natural history of APS and risk predictors for thrombosis (Galli et al., 2003b). However, as the aPL status may change over time, they also carry the risk of underestimating or overestimating the association with thrombosis (Galli et al., 2003b). When an association is determined, it is typically indicative of a stable condition (Galli et al., 2003b). Finally, retrospective studies provide limited information not

only because of a lack of controls but because they cannot determine whether aPL are causative, coincidental, or a result of clinical events (Finazzi, 2001). These studies may also be subject to ascertainment bias (those with history of thrombosis are more likely to have assessment for risk factors such as aPL) and referral bias, possibly leading to falsely overestimated risks (Somers et al., 2002). In addition, retrospective studies often assess exposures at one time point, but patients, such as those with SLE and aPL, can have fluctuating titers over time (Somers et al., 2002).

In addition to the challenges of gathering epidemiological data on APS related to the limitations posed by various study designs, there is controversy surrounding the best way to determine aPL positivity (Finazzi, 2001). The initial observation that phospholipid extracted from beef heart (cardiolipin) was a major antigen for the reagin in a syphilis assay prompted the development of the aCL enzyme-linked immunosorbent assay (ELISA) used in the diagnosis of APS patients today (Pangborn, 1941; Moore and Mohr, 1952). The subsequent discovery of the LA in patients with SLE (Conley and Hartman, 1952) was later correlated with pregnancy morbidity (Beaumont, 1954) and thrombosis (Bowie et al., 1963). In the 1990s it was finally determined that aPL recognize phospholipid-binding proteins, and that anti-β_2GPI was clinically the most important one (Bas de Laat et al., 2004a). Currently, there are assays that have demonstrated a variable but fair association with thrombosis when used in prospective studies, including phospholipid-dependent coagulation assays, anti-β_2GPI ELISA-based assays and aCL ELISA-based assays (Bas de Laat et al., 2008).

Although we have made advances in the laboratory detection of aPL and aPL-associated binding proteins since the last century, there is considerable variation among the different types, isotypes, cut-off values, and laboratory methods used in detecting them (Galli et al., 2003b). It is important to have highly specific diagnostic assays to detect aPL, as reflected in the laboratory criteria for the confirmation of definite APS (Wilson et al., 1999; Miyakis et al., 2006). Unfortunately, despite attempts to make serological criteria more specific with the revised 2006 classification criteria, many patients are still incorrectly diagnosed and, as a result, inadequately treated (Wilson et al., 1999; Galli et al., 2003a; Miyakis et al., 2006).

There are still many inconsistencies between different laboratories and assays on the measurement of aCL (Wong, 2004). A study by Favaloro and Silvestrini found an interlaboratory coefficient of variation for aCL IgM and IgG of over 50% (Favaloro and Silvestrini, 2002). The factors contributing to the variation in results include the use of in-house versus commercial aCL ELISA kits, calibration of the assays, and the source and purity of the antigens (Wong, 2004). In addition, the aCL assay is generally very sensitive but not very specific, being positive in about 80% of patients with APS (Bertolaccini and Khamashta, 2006; Bas de Laat et al., 2008). This low specificity is explained by the fact that aCL can also be falsely positive in other disorders where their presence is not associated with thrombosis, such as infectious diseases and connective tissue diseases (Mouritsen et al., 1989). It is usually IgM aCL in the low-positive range that gives false-positive results in these other disorders (Miyakis et al., 2006). Finally, it has been demonstrated that many aCL target the antigen β_2GPI (Bertolaccini and Khamashta, 2006). Hence, differences between serum concentrations of β_2GPI in patients and in the assay reagents may also influence the results of different aCL assays (Wong, 2004).

The updated 2006 consensus statement for the diagnosis of APS has included IgG and IgM anti-β_2GPI antibodies as an independent risk factor for thrombosis and pregnancy complications (Miyakis et al., 2006). The interlaboratory variation and specificity of anti-β_2GPI is better than found with the aCL assay, suggesting that positivity in the anti-β_2GPI assay is more correlated with clinical manifestations of APS than positivity in conventional aCL ELISAs (Bertolaccini and Khamashta, 2006; Miyakis et al., 2006). There is even evidence that in 3–10% of patients with APS, anti-β_2GPI may be the only positive test (Lee et al., 2003a). However, similar to the aCL assays, methodological differences and lack of standardization still exist (Reber et al., 2002; Galli et al., 2003a). Investigators of the European Forum on

Antiphospholipid Antibodies formed a collaborative study to analyze the interlaboratory variability of anti-β_2GPI assays (Reber et al., 2002). They sent 28 samples from patients to 21 European centers for analysis, and found a large variability of anti-β_2GPI measurements between centers, with only 13% of cases for IgG and 6% of cases for IgM showing excellent concordance (Reber et al., 2002). The investigators attributed the high variability to many low-positive samples, and reported good agreement on results with samples with medium or high positivity (Reber et al., 2002).

Generally, LA assays are more specific and less sensitive than aCL for APS, with LA positivity found in about 20% of patients with APS (Bertolaccini and Khamashta, 2006). As with the other aPL, interlaboratory agreement is fairly poor for most LA assays currently available, and there is no evidence that one assay correlates with clinical events of APS better than another (Miyakis et al., 2006). However, in patients with autoimmune diseases, there is evidence that β_2GPI-dependent LA is strongly associated with a history of thrombosis, in contrast to the β_2GPI-independent LA assay (Bas de Laat et al., 2004b). Bas de Laat and colleagues evaluated the association between anti-β_2GPI-dependent LA and vascular thrombosis in a cross-sectional study of 198 patients with SLE ($n = 176$), lupus-like disease ($n = 16$), and primary APS ($n = 6$) (Bas de Laat et al., 2004b). Presence of antibodies to β_2-GPI-dependent lupus anticoagulant was highly associated with a history of thromboembolic complications (odds ratio 42.3; 95% confidence interval 9.9–194.3) (Bas de Laat et al., 2004b). These findings support other studies suggesting that antibodies to β_2GPI with LA activity are responsible for the thromboembolic complications in APS (Bas de Laat et al., 2004b). Using the same patient cohort, Bas de Laat and colleagues subsequently published another study and retrospectively determined that, specifically, IgG antibodies recognize epitope Gly40–Arg43 in domain I of β_2GPI and have LA activity that correlates strongly with thrombosis (Bas de Laat et al., 2005).

The value of IgA aPL and their association with thrombosis and pregnancy morbidity are still controversial (Fanopoulos et al., 1998; Bertolaccini et al., 2001; Lee et al., 2001; Carmo-Pereira et al., 2003; Samarkos et al., 2006). The revised 2006 classification criteria for APS recognized that the IgA isotype identifies particular APS subgroups, however concluded that data are inadequate for making IgA anti-β_2GPI an independent risk factor for APS in the absence of IgG and/or IgM anti-β_2GPI (Miyakis et al., 2006). There is evidence to support a high prevalence of IgA aCL and IgA anti-β_2GPI in African-Americans with SLE compared with other ethnic groups (Cucurull et al., 1999; Diri et al., 1999). Molina and colleagues studied 152 African-American, 136 Afro-Caribbean (Jamaican), and 163 Hispanic (Colombian) unselected patients with SLE, and reported that their main finding was the higher prevalence of IgA aCL in the Afro-Caribbean population (21%), with IgA being the only isotype detected in 82% of these patients (Molina et al., 1997). However, the IgA isotype was not correlated with any clinical manifestations of APS and was typically found in low titers (Molina et al., 1997). In a series by Diri and co-workers of eight African-American patients with APS, IgA was not only the most frequent isotype of aCL and anti-β_2GPI, but it co-occurred with the IgM isotype in three of the four patients with neurological manifestations (Diri et al., 1999). In another analysis of 418 consecutive patients with SLE or APS by Danowski and colleagues, both IgM and IgA were the most frequent isotypes of anti-β_2GPI (Danowski et al., 2006). However, there was a significantly higher prevalence of the IgM isotype seen in white patients (21.8%) compared with African-Americans (8.1%), with a p-value of 0.0008 (Danowski et al., 2006). Petri and colleagues found a higher prevalence of LA (by dilute Russell's viper venom time; dRVVT) and aCL in white than in African-American patients with SLE (Petri et al., 1991).

In conclusion, progress in the standardization of measurement of aPL has been made. However, there is clearly a need for development of more specific laboratory assays to capture those patients at particular risk of thrombosis or pregnancy morbidity (Bertolaccini and Khamashta, 2006). From these data and future investigations, prevalence of aPL in different ethnic groups and their clinical significance in APS can be determined.

12. Antiphospholipid antibodies in systemic lupus erythematosus and other diseases

Systemic lupus erythematosus (SLE) is the main autoimmune disease associated with the APS (Levine et al., 2002). In general, the prevalence of aPL among patients with SLE ranges from 12% to 44% for aCL, from 15% to 34% for LA antibodies, and from 10% to 19% for β_2GPI antibodies (Alarcon-Segovia et al., 1989; Love and Santoro, 1990; Wong et al., 1991; Cervera et al., 1993; Merkel et al., 1996; Petri, 2000; Tubach et al., 2000). However, cross-sectional studies have been known to underestimate the true frequency of aPL in SLE, as many SLE patients make these antibodies intermittently or only after thrombotic events (Love and Santoro, 1990; Petri, 2000). For instance, there is evidence for variation in levels of both aCL and LA in patients tested every 3 months in the Hopkins Lupus Cohort, with higher levels of aPL reported at times of disease activity and lower levels if the patient is treated with prednisone and/or hydroxycholoroquine (Petri, 2000). Gomez-Pacheco and colleagues assessed the prevalence and levels of antibodies to β_2GPI and to cardiolipin in SLE patients before, during, and after thrombosis compared with SLE patients without thrombosis (Gomez-Pacheco et al., 1999). The prevalence and levels of aCL were similar in SLE patients with and without thrombosis (Gomez-Pacheco et al., 1999). All patients with thrombosis were found to have anti-β_2GPI, compared with only 17% of controls ($p < 0.0001$) (Gomez-Pacheco et al., 1999). Furthermore, a significant decrease in serum anti-β_2GPI levels was observed at the time of thrombosis, as compared with before and after the event (Gomez-Pacheco et al., 1999).

Evidence has shown that APS may develop in 30–70% of patients with SLE and aPL after 20 years of follow-up (Love and Santoro, 1990; Alarcon-Segovia et al., 1992; Petri, 2000). A meta-analysis by Wahl and colleagues reviewed 26 publications to determine the association between aPL and VTE in SLE, and found that patients with LA have a sixfold greater risk for VTE than patients without LA, whereas patients with aCL have a twofold greater risk for VTE than patients without aCL (Wahl et al., 1997). However, up to 30% of patients with SLE and aCL have demonstrated no clinical manifestations of APS over an average follow-up of 7 years (Alarcon-Segovia et al., 1992).

Love and Santoro performed a meta-analysis of 29 published series and evaluated the prevalence and clinical significance of aPL in SLE (over 1000 patients) and non-SLE disorders (Love and Santoro, 1990). The authors reported that in six series where the underlying diseases of consecutive LA-positive patients were recorded, only 35% had SLE or lupus-like disease (Love and Santoro, 1990). Of the remaining patients, 9% had another autoimmune disorder, 12% had drug-induced LA, 3% had cancer, and 41% had other miscellaneous or unspecified disorders (Love and Santoro, 1990). In studies examining the association of aCL in non-SLE disorders, there was no correlation with thrombosis (Love and Santoro, 1990). For instance, only 10% of aCL-positive patients compared with 6% of aCL-negative patients with rheumatoid arthritis had thrombosis (Love and Santoro, 1990).

Merkel and colleagues determined the prevalence and clinical associations of aCL in a 5-year, blinded, controlled, prospective study of patients with a variety of connective tissue diseases enrolled in a Cooperative Study of Systematic Rheumatic Diseases (CSSRD), supported by the National Institutes of Health (Merkel et al., 1996). The investigators found the prevalence of either IgG or IgM aCL among each diagnostic group as the following: SLE 15.76%, rheumatoid arthritis (RA) 15.7%, scleroderma (PSS) 6.7%, polymyositis/dermatomyositis (PM/DM) 8.3%, early undifferentiated connective tissue disease (EUCTD) 9.1%, Sjögren's syndrome (SJ) 6.8%, ANCA-related renal vasculitis 3.8%, and blood bank controls 4% (Merkel et al., 1996). The prevalence of aCL was significantly higher in patients with RA or SLE compared with controls ($p < 0.01$), however, the prevalence of the rest of the connective tissue diseases approached that of the general population (Merkel et al., 1996). The prevalence of aCL in this cohort of patients with RA, SLE, and scleroderma were generally lower in comparison to previous reports of frequencies for these diseases (4–49%,

12–39%, and 0–41%, respectively) (Manoussakis et al., 1987; Font et al., 1989; Passaleva et al., 1990; Fonollosa et al., 1991; Merkel et al., 1996; Petri, 2000; Sanna et al., 2005). Very few consistent clinical associations among patients with connective tissue diseases and aPL were found, such as hemolytic anemia and positive serologic test for syphilis (Merkel et al., 1996). Thus, the investigators of the CSSRD cohort concluded that the clinical importance and specificity of these antibodies has yet to be determined (Merkel et al., 1996).

Another group determined the prevalence and clinical significance of aCL, anti-β_2GPI and antibodies to phosphatidylserine–prothrombin complex (aPS-PT) in a series of 25 patients with scleroderma (Sanna et al., 2005). aCL IgG and/or IgM were more frequently found in patients with scleroderma than in controls (24% versus 5%, respectively, $p = 0.0008$), while the prevalence of anti-β_2GPI did not differ between the two groups (Sanna et al., 2005). Scleroderma patients with telangiectasias and pulmonary hypertension had a higher prevalence of IgM aPS-PT than controls (37.5% vs. 0%, $p = 0.02$ and 66.6% vs. 4.5%, $p = 0.03$, respectively) (Sanna et al., 2005). The authors noted that the association of aPS-PT with vascular complications in scleroderma deserves further investigation but concluded that, although aPL were commonly detected in these patients, their association with clinical manifestations of APS was not frequently seen (Sanna et al., 2005).

The presence of aPL has also been detected in various non-autoimmune conditions such as infections, cancer, and with the use of certain drugs (such as chlorpromazine, procainamide, hydralazine, phenytoin, quinidine, interferon, and cocaine) (Mouritsen et al., 1989; Love and Santoro, 1990; Schved et al., 1994; Asherson, 2000). However, the aPL associated with these other conditions are typically low-titer IgM antibodies that are not associated with thrombosis or other aPL-related clinical features (Bertolaccini and Khamashta, 2006). In Love and Santoro's meta-analysis of aPL, a lower frequency of thrombosis was evident in LA-positive patients with non-SLE disorders (22%) compared with LA-positive patients with SLE (42%) (Love and Santoro, 1990).

Estimates of the frequency of aPL in patients with non-autoimmune related diseases vary, as is the case with malignancy-associated aPL (Asherson, 2000). aPL have been associated with a variety of malignancies, including solid tumors, as well as lymphoproliferative and hematological malignancies (Asherson, 2000). The Montpellier Antiphospholipid (MAP) Study, a prospective epidemiological study on the occurrence of aPL, evaluated 1014 patients admitted to a department of internal medicine (mean age 66.7 years) for aPL (Schved et al., 1994). The prevalence of aPL in their large series of patients was 7.1% ($n = 72$), and malignancy was determined to be the most frequently associated disease (Schved et al., 1994). Fourteen of the 72 aPL-positive patients had active malignancy ($n = 7$) or a history of cancer ($n = 7$) (Schved et al., 1994). Zuckerman and colleagues studied the prevalence and clinical significance of aCL in 216 patients with malignancy and 88 healthy control subjects (Zuckerman et al., 1995). Forty-seven (22%) of the patients with malignancy were found to be aCL-positive compared with only 3 (3%) of the control group (Zuckerman et al., 1995). The aCL-positive cancer patients had a higher rate of thrombosis than the aCL-negative cancer patients (28% vs. 14%, respectively, $p < 0.05$) (Zuckerman et al., 1995). Levels of aCL decreased in four of the patients after successful treatment of the malignancy and were persistently negative, with no occurrence of thromboembolic events during 1 year of follow-up (Zuckerman et al., 1995). The investigators demonstrated a higher prevalence of aPL in patients with malignancies, as well as an association between thrombosis and aPL in this population (Zuckerman et al., 1995). However the interplay between malignancy, development of aPL, and thrombosis is still poorly understood (Zuckerman et al., 1995).

In addition to autoimmune diseases and malignancy, increased levels of aPL have also been associated with a variety of infectious diseases (Cervera et al., 2004). The clinical features typical of APS are not commonly observed with aPL-associated infections (Asherson and Cervera, 2003), with aCL of the IgM isotype found more frequently than the IgG isotype (Bertolaccini and Khamashta, 2006). The aPL found in infections

can be distinguished from those found in APS by determining LA activity and anti-β_2GPI, as these antibodies are related to the thrombotic features of APS but are not present in most cases of infection (β_2GPI-independent or non-thrombogenic aPL) (Amin, 2008). However, there is evidence that some infections are associated with aPL that possess anti-β_2GPI activity and are more likely to cause thrombotic complications (β_2GPI-dependent), such as leprosy and parvovirus B19 (Asherson and Cervera, 2003). Infection, more commonly viral, has also been implicated in development of aPL that trigger the clinical manifestations of catastrophic APS in as many as 40% of cases (Asherson and Cervera, 2003). Some of the infectious diseases that have been demonstrated to precede CAPS involve the respiratory tract (15%), skin (8%), and urinary tract (6%) (Asherson and Cervera, 2003). Similarly, Cervera and colleagues analyzed 100 patients with APS associated with infections, and found the main associated infections included skin infection (18%), human immunodeficiency virus (17%), pneumonia (14%), hepatitis C (13%), and urinary tract infection (10%) (Cervera et al., 2004).

Many viral infections have demonstrated the presence of aPL, but they are not typically associated with clinical features of APS. The prevalence of aPL in hepatitis C has been reported to range from 3.3% to 46% (Dalekos et al., 2001), and is typically unrelated to manifestations of APS (Amin, 2008). The prevalence of aCL in patients with human immunodeficiency virus (HIV) has been reported to be even higher, with a range of 12–67% (de Larranga et al., 1999; Amin, 2008). However, anti-β_2GPI are rarely found in HIV patients and the frequency of aPL-related thrombotic complications remains low (Abuaf et al., 1997; Amin, 2008). A high prevalence of IgG aCL (56%) has been shown on three-month follow-up of patients with cytomegalovirus (CMV) infection who received unrelated bone marrow and cord blood allogenic stem cell transplantation for hematologic malignancies (Mengarelli et al., 2000). Similar to other viral diseases such as hepatitis C and HIV, no association with features of APS was noted with CMV and the presence of aPL (Amin, 2008). Finally, aPL have been detected in patients with other viral infections as well, including varicella virus, human T-lymphotropic virus type 1, and Epstein–Barr virus (Amin, 2008).

aPL have also been seen in various bacterial infections. However, increases in aPL are usually β_2GPI-independent and not typically associated with thrombotic events (Amin, 2008). The prevalence of aCL (mainly of the IgG and IgM isotype) has been reported for various bacterial diseases, such as leprosy (33–67%), tuberculosis (27–53%), bacterial endocarditis (5–44%), *Mycoplasma* (20–53%), *Streptococcus* (0–80%), *E. coli* (67%), *Salmonella* (60%), *Staphylococcus aureus* (43%), *Leptospirosis* (50%), *Borrelia burgdorferi* (14–41%), Malaria (30%) and *Coxiella burnetii*, the bacterial organism responsible for Q fever (42–84%) (Blank et al., 2004).

13. Longitudinal studies

Longitudinal studies of the antiphospholipid syndrome are very important, as they can examine the association between aPL and other risk factors with subsequent morbidity and mortality related to thrombosis. By comparing these associations of aPL in patients with APS to people in the general population, we can gain a better understanding of the epidemiological aspects regarding APS.

The Euro-Phospholipid Project is a multicenter study that has prospectively followed a cohort of 1000 consecutive patients with APS since 1999 (Cervera, 2008). The investigators of the Euro-Phospholipid Group reported that overall, the prevalence of the major clinical features accepted as classification criteria (Wilson et al., 1999; Miyakis et al., 2006) found in their cohort were similar to those reported in previous studies (Alarcon-Segovia et al., 1989; Mackworth-Young et al., 1989; Font et al., 1991). Very common manifestations were DVT (38.9%), stroke (19.8%), PE (14.1%), transient ischemic attacks (11.1%), and MI (5.5%) (Cervera et al., 2002).

In another longitudinal study involving the validation of the 2006 revised APS classification criteria, 200 aPL-positive patients were analyzed (Kaul et al., 2007). Approximately 50% of the

patients in this cohort with vascular events had at least one identifiable non-aPL thrombosis risk factor at the time of their vascular events (Kaul et al., 2007). Similar results were demonstrated in another prospective study of 404 patients (APS and asymptomatic carriers of aPL), where at the time of the first thrombotic event about half of the patients had coincident non-aPL risk factors for thrombosis (Giron-Gonzalez et al., 2004). Giron-Gonzalez and colleagues also examined the location of thrombotic episodes in patients with primary APS, and found that the lower extremities were the most common site of venous thrombosis (82.9%) whereas the central nervous system, including the retina, was the most frequent site of arterial thrombosis (71%) (Giron-Gonzalez et al., 2004).

Previous prospective studies have identified high aCL levels as an independent risk factor for atherosclerotic vascular diseases, mainly MI, stroke, and peripheral occlusion (Vaarala et al., 1995; Turiel et al., 2005). In a 4-year prospective study of 360 unselected patients with aPL, Finazzi and colleagues identified previous thromboses and a high titer of IgG aCL (>40 GPL units) as independent predictors of new thrombotic episodes (Finazzi et al., 1996). In their study, patients with a history of vascular events had further complications at a rate of 5.4% patient-years, compared with 0.95% patient-years in asymptomatic individuals (Finazzi et al., 1996). Turiel and investigators performed a 5-year prospective study of 56 patients with APS and found a correlation between potential embolic sources and high aCL titers, with an incidence of 43.3% new thrombotic events in patients with IgG aCL levels of >40 GPL units compared with 7.7% in those with less than or equal to 40 GPL units (Turiel et al., 2005). Finally, Ginsberg and colleagues reported that a high titer of aCL was a risk factor for DVT or pulmonary embolus in a prospective cohort of healthy men, independent of age and smoking status (Ginsberg et al., 1992).

The Hopkins Lupus Cohort is a longitudinal study in which aPL (LA by dRVVT and aCL by polyclonal ELISA) are measured on a quarterly basis (Somers et al., 2002). Somers and colleagues evaluated aPL and the incidence of venous thrombosis in 678 patients from the Hopkins Lupus Cohort, and estimated that SLE patients with the LA had a 42% chance of developing venous thrombosis within 20 years of diagnosis (Somers et al., 2002). However, no significant value of aCL was found as an independent risk factor for venous thrombosis during follow-up (ranging from 0 to 14 years, with 44% patients observed in the cohort for more than 5 years) (Somers et al., 2002). In another study of 139 patients with SLE, although IgM aCL was shown to be negatively correlated with survival, thromboembolic events were not a major cause of death in the cohort (Gulko et al., 1993). Finally, a study of 37 SLE patients with LA and 37 matched SLE patients without LA were followed for a median of 22 years (Jouhikainen et al., 1993). DVT occurred in 54% of LA-positive SLE patients, and in most cases the events occurred within the first 8 years (Jouhikainen et al., 1993).

Longitudinal data from the European Multicenter Study has shown that patients with APS (average follow-up 2 years) may develop further thrombotic events despite anticoagulation therapy (Vianna et al., 1994). Rosove and Brewer studied 70 APS patients with arterial or venous thrombosis (mean follow-up of 5.6 years), and determined that 53% of patients had recurrent thrombotic events (Rosove and Brewer, 1992). The risk of recurrent thrombosis was high if anticoagulation was not maintained (Rosove and Brewer, 1992). In another study, the association of aPL with the development of clinical thrombotic manifestations and the efficacy of anti-thrombotic therapies used were analyzed in 272 SLE patients with or without APS (Tarr et al., 2007). During a 5-year follow-up period, a higher frequency of new thrombotic events occurred in aPL-positive patients not on treatment compared with aPL-positive patients receiving primary prophylaxis (Tarr et al., 2007). The findings from these longitudinal studies and other prospective studies (Schulman et al., 1998; Crowther et al., 2003; Finazzi et al., 2005), demonstrating that APS patients with thrombosis will be subject to recurrences, emphasizes the importance of identifying those patients at risk for thrombosis in order to provide them with adequate long-term anticoagulation (Bertolaccini

and Khamashta, 2006). However, the optimal international normalized ratio (INR) of warfarin is still unknown for patients with aPL-associated thrombosis (Khamashta and Hunt, 2005).

The Antiphospholipid Syndrome Collaborative Registry (APSCORE) is a prospective cohort study that has recently been developed for patients with APS. Researchers and clinicians will hopefully use this registry as a resource to support more epidemiological studies of APS.

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CHAPTER 3

Laboratory Heterogeneity of Antiphospholipid Antibodies

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1. The antiphospholipid syndrome: definition and history

The antiphospholipid syndrome (APS) is an autoimmune multisystemic disorder of recurrent thrombosis and/or pregnancy losses that is associated with the presence of antiphospholipid (aPL) antibodies (Harris, 1987). APL antibodies are a heterogenous group of autoantibodies directed against anionic phospholipids or protein–phospholipid complexes, measured in solid-phase immunoassays such as anticardiolipin (aCL) or as an activity (functional assays) which prolongs phospholipid-dependent coagulation assays, the so-called lupus anticoagulants (LAC) (Triplett and Brandt, 1988; McNeil et al., 1990; Pierangeli et al., 1992; Roubey et al., 1995).

The first description of aPL antibodies dates back to 1952, when Moore and colleagues described patients with systemic lupus erythematosus (SLE) with a persistently false-positive VDRL flocculation test for syphilis, a test based on the detection of antibodies against cardiolipin—also named diphosphatidylglycerol—extracted from beef heart (Moore and Mohr, 1952). In the same year, Conley et al. described two SLE patients with a peculiar circulating inhibitor of coagulation (Conley and Hartmann, 1952). It came to be understood that these anticoagulants could inhibit in vitro coagulation assays, but did not inhibit individual coagulation factors, and were not associated with a bleeding diathesis unless other coagulopathy was present. Feinstein and Rapaport introduced the term LAC to describe this phenomenon in 1972 (Feinstein and Rapaport, 1972). Although the relation between thrombosis and the presence of these anticoagulants in SLE patients was already noticed in 1963, it was not until 1980 that the association between LAC and thrombosis was widely recognized (Bowie et al., 1963; Mueh et al., 1980). The association of LAC with false-positive syphilis tests led to the development of a quantitative immunoassay for aCL and the establishment of the association between thrombosis and aCL antibodies (Harris et al., 1983). Thereafter, patients presenting with thrombosis and/or pregnancy loss in combination with persistently positive aCL antibodies and/or circulating LAC were deemed to have the new diagnosis of the "anticardiolipin syndrome," which then became named the APS (Gharavi et al., 1987). Although initially described in patients with SLE, APS was soon recognized to also occur in patients without underlying autoimmune disease (Harris et al., 1988). APS was then classified as "secondary" SAPS in the presence of SLE, and "primary" APS in the absence of SLE or other autoimmune disorders (Rand, 2003).

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Primary APS is the most common cause of acquired thrombophilia and accounts for 15–20% of all episodes of deep vein thrombosis with or without pulmonary embolism, one-third of new strokes occurring in patients under the age of 50, and 10–15% of women with recurrent fetal loss (Ginsburg et al., 1992). It has been estimated that 2–5% of the general population have experienced an episode of deep vein thrombosis, suggesting that the prevalence of venous thrombosis associated with primary APS may be as high as 0.3–1% of the general population (Ginsburg et al., 1992). APS also accounts for a significant proportion of thromboembolic disease and recurrent fetal loss in patients with SLE (Tincani et al., 2003). APL antibodies are present in 30–40% of SLE patients, and approximately one-third of those with antibodies, or 10–15% of all SLE patients, have clinical manifestations of APS (Levine et al., 2002).

Besides the well-known association with a thrombophilic state, aPL antibodies have been also related to recurrent pregnancy loss and obstetric complications such as preeclampsia (Sailer et al., 2006). It is now generally accepted that aPL antibodies are the most frequent acquired risk factor for a treatable cause of recurrent pregnancy loss and for pregnancy complications (early and severe preeclampsia) (McClain et al., 2004).

Although many researchers took an early interest in the syndrome, consensus classification criteria to assist patient inclusion in clinical studies were lacking until recently. It was not until 1999 that an international consensus meeting formulated the first classification criteria (the ''Sapporo criteria'') for patients with the APS, contributing greatly to the value of experimental data obtained from population studies (Wilson et al., 1999). Since then, these criteria have been updated in 2004 at another international consensus meeting (the ''Sydney criteria'') (Miyakis et al., 2006). According to the original 1999 criteria, definite diagnosis of APS requires the presence of one of two major clinical manifestations of APS (pregnancy loss or thrombosis) together with positivity in one of the two major laboratory tests (aCL or LAC) on at least two occasions. Initially, the 1999 Sapporo criteria did not include other tests frequently used in APS, such as anti-β_2 glycoprotein I (anti-β_2GPI). Subsequently, and during a preconference workshop at the XIth International Congress on Antiphospholipid Antibodies in November 2004 in Sydney, Australia, it was decided by general consensus that the detection of anti-β_2GPI should be added to the 1994 Sapporo classification criteria (Miyakis et al., 2006). This was based on reports that this test may be found to be positive in a small percentage of patients with APS who were negative for aCL and LAC, as discussed in previous sections.

2. Serological assays used in the diagnosis of antiphospholipid syndrome

2.1. The aCL test: characteristics and standardization

The aCL test was originally described in 1983 as a radioimmunoassay and was subsequently modified and standardized (Harris et al., 1983; Gharavi et al., 1987). The aCL assay is simple, sensitive, and provides helpful information to aid in the diagnosis of APS. In addition, a positive aCL test is important in the diagnosis of APS, particularly since clinical features of the disorder can occur in many other disorders, including hereditary thrombophilias. While an isolated positive result in the absence of clinical manifestations (such as thrombosis or fetal loss) does not support a diagnosis of the syndrome (Wilson et al., 1999; Miyakis et al., 2006), a positive result in the presence of these clinical manifestations does support a diagnosis of APS. Moreover, it is generally accepted that the test must be positive on at least two occasions 12 or more weeks apart to confirm the diagnosis (Miyakis et al., 2006).

Several attempts have been made to standardize the aCL test, including several international workshops and a European forum specifically convened for this purpose. Work undertaken by several national and international organizations including the US College of American Pathologists and the UK-based National External Quality Assessment Scheme (NEQAS) has also contributed to the

standardization of the aCL test (Harris et al., 1987, 1994; Harris, 1990a, b; Reber et al., 1995; Pierangeli et al., 1998). Results from the surveys sponsored by those organizations were discordant. For example, while results from some of the European programs are discouraging and less than satisfactory—not only for the aCL but also for the anti-β_2GPI assay—a much better performance and interlaboratory agreement may be appreciated in surveys hosted by the College of American Pathologists. The College enrolls certified laboratories in quality control surveys for aPL testing and requires participation as part of the Laboratory Accreditation Program (LAP). As shown in Table 1, the overall performance and interlaboratory agreement of aCL and anti-β_2GPI assays may be considered from very good to excellent when analyzing data of all aPL surveys from 2004 to 2008. Importantly, the only discrepancies were observed in few samples that had values in the low positive–borderline range.

In a study by Erkan et al., the investigators, puzzled by the challenges frequently faced by clinicians interpreting routine laboratory results, examined the stability and the degree of variation of aPL values in a real-world setting in which aCL tests are done in multiple laboratories. The authors reviewed clinical characteristics, medications, and 1652 data points concerning aCL and other laboratory testing of 204 aPL-positive patients. For the aCL tests, the data were obtained using either an in-house assay (Erkan et al., 2005) or one of two commercial kits (INOVA and Pharmacia). Seventy-five per cent of initial moderate to high positive aCL results remained in the same range regardless of the laboratory performing the test. On same-day specimens, the consistency of aCL results between suppliers ranged from 64% to 88% and the correlation ranged from 0.5 to 0.8. Agreement was moderate for aCL IgG and for aCL IgM. However, for aCL IgA, agreement was only marginal. The study also showed that the interlaboratory agreement was better when positive aCL results were compared in a semi-quantitative manner (ranges of positivity), as previously demonstrated (Erkan et al., 2005). The authors concluded that aCL results remained stable in at least three-quarters of subsequent tests regardless of the laboratory performing the test.

Despite these efforts, a considerable degree of interlaboratory variation has been reported (De Moerloose et al., 1990; Peaceman et al., 1992; Reber et al., 1995; Tincani et al., 2001; Favaloro and Silvestrini, 2002; Harris and Pierangeli, 2002; Wong et al., 2004). Any number of factors can contribute to the variation in results from in-house and commercial aCL ELISAs. These include pre- and post-analytical factors, as well as issues related to the manufacture and calibration of the assays, differences in the microtiter plates or the way cut-off points are established (De Moerloose et al., 1990; Reber et al., 1995; Pierangeli et al., 2001; Favaloro and Silvestrini, 2002; Kutteh and Franklin, 2004). Thus, aCL assays (both in-house and commercial) face problems common to all auto-antibody assays, including source and purity of the

Table 1
Summary of College of American Pathologists surveys for anticardiolipin and anti-β_2 glycoprotein I assays from 2004 to 2008

Test	No. of samples evaluated	No. of samples graded	No. of samples not graded due to lack of consensus	No. of different kits/ assays evaluated	Total no. of responses
aCL IgG	27	23	4[a]	18–23	1872
aCL IgM	27	24	3[a]	17–22	1632
aCL IgA	27	16	11[a]	12–16	906
Anti-β_2GPI IgG	27	26	1[a]	10–12	463
Anti-β_2GPI IgM	27	27	0	9–11	408
Anti-β_2GPI IgA	27	27	0	6–8	285

Note: Two shipments of three freeze-dried samples are sent to participating laboratories each year.
[a] Results of the samples returned to the College of American Pathologists are scored if qualitative and quantitative consensus (>90% agreement) is reached among participants. Assays evaluated: commercial and in-house aCL and anti-β_2GPI (IgG, IgM, IgA).

antigens, specificity of detection antibodies, and the isotype and heterogeneous avidity spectrum of antibodies detected. It is believed that most of the problems encountered may be resolved by consistently applying good laboratory practice in the routine clinical laboratories and by fully implementing good manufacturing practice by commercial companies that manufacture kits.

The importance of aCL titers has also been addressed. Based on work done by several groups, thrombosis, recurrent fetal loss, and thrombocytopenia appear to occur more frequently as the level of aCL increases. An aCL titer of >35–40 GPL units has been associated with an increased risk of thrombosis. In addition, the IgG isotype appears to be more closely associated with clinical manifestations than either the IgM or IgA isotypes (Harris et al., 1986; Gharavi et al., 1987; Escalante et al., 1995; Silver et al., 1996; Levine et al., 1997). Studies by Levine and Escalante and their respective colleagues demonstrated that medium and high levels of aCL antibodies are more significantly correlated with the clinical manifestations of APS (Escalante et al., 1995; Levine et al., 1997). According to Escalante et al., IgG aCL levels below 21.4 had a low (0.07) probability of thrombosis, while IgG aCL levels between 21.4 and 65.0 GPL had an increased probability of thrombosis of 0.20, and when aCL IgG values were greater than 65 GPL units they were associated with an even higher probability value of 0.75 (Escalante et al., 1995).

Low levels of aCL antibodies are also present in several other disorders. Hence, the specificity of the aCL test for APS also tends to be lower when the aCL values are low positive. Recent reports show an apparently large number of individuals with low to moderate titers of aCL antibodies, particularly of the IgM isotype, with no clinical signs of APS. Physicians are frequently puzzled with respect to the clinical significance of such results. In order to clarify this issue, our group carried out a study in which the prevalence of positive IgM aCL antibodies was determined in a large cohort of normal healthy individuals and elderly people (total 1141 samples) using three different IgM aCL assays (two commercial kits and an in-house assay). ACL levels at 95th and 99th percentiles were calculated. The cut-offs calculated at the 95th percentile showed that the currently accepted values are correct for the three assays. It was also found that the vast majority of the IgM aCL positive samples in this large population of healthy individuals fell between the 95th and the 99th percentile. Subsequently, a similar evaluation of the cut-off values and the meaning of low positive results was done in two of the three aPL assays for IgG and IgA isotypes. Results are similar to what was seen with the IgM isotype and are depicted in Tables 2 and 3. Based on this study,

Table 2
Reappraisal of the cut-off and low positive ranges for the anticardiolipin ELISA (IgG, IgM, and IgA)

$N = 1141$	aCL IgG (GPL units)	aCL IgM (MPL units)	aCL IgA (APL units)
95th percentile (lower limit of indeterminate zone)	6.9	5.5	13.9
99th percentile (upper limit of indeterminate zone)	18.7	14.8	27.4
Current stated normal cut-off	<10	<10	<15

Source: From Budd et al. (1996).

Table 3
Reappraisal of the cut-off and low positive range for the APhL ELISA (IgG and IgM)

$N = 1141$	APhL IgG (GPL units)	APhL IgM (MPL units)
95th percentile (lower limit of indeterminate zone)	13.9	10.6
99th percentile (upper limit of indeterminate zone)	27.4	38.4
Current stated normal cut-off	<15	<15

Source: From Budd et al. (1996).

it was proposed that the range of values between the 95th and the 99th percentile be considered (or reported) as ''indeterminate'' (Budd et al., 2006). It was also recommended that samples falling in this category be retested at a later date (with the current recommendation being 12 weeks, as per the revised Sydney APS criteria) to confirm persistence of positivity (Budd et al., 2006). However, given the likelihood that APS is a disease with more than one autoantibody and that these autoantibodies may vary in specificity, a low positive aCL test result need not exclude the presence of other pathogenic antibodies. It is now clear that sera from patients with APS contain a mixture of antibodies specific for β_2GPI, negatively charged phospholipids, and possibly other proteins such as prothrombin (PT), protein C, protein S, and others.

2.2. More specific serological assays for antiphospholipid syndrome

Although a sensitive test, aCL ELISA tests are positive in a variety of disorders, including connective tissue diseases, infectious disorders (including, but not limited to, syphilis, Lyme disease, Q fever, hepatitis C, tuberculosis, leprosy, and HIV) and some drug-induced disorders (Canoso and Sise, 1982; Canoso et al., 1987; Gharavi et al., 1987; Harris et al., 1988; Intrator et al., 1988; Mouritsen et al., 1989; Gálvez et al., 1997).

Current studies show that β_2GPI, particularly when coated on oxidized or ''high-binding'' polystyrene ELISA plates, is a relatively specific antigen for autoantibodies present in APS patients (Arvieux et al., 1992; Viard et al., 1992; Matsuura et al., 1994; Balestrieri et al., 1995; Cabiedes et al., 1995; Martinuzzo et al., 1995; Roubey et al., 1995; Katano et al., 1996; Ogasawara et al., 1996; Tsutsumi et al., 1996; Lewis et al., 1998). Anti-β_2GPI have been reported to be associated primarily with thrombosis in patients with APS, but other studies have shown also these antibodies in patients with pregnancy loss and other manifestations of APS (Matsuura et al., 1994; Martinuzzo et al., 1995; Ogasawara et al., 1996; Tsutsumi et al., 1996). A meta-analysis by Galli et al. addressed the value of anti-β_2GPI test as a risk factor for thrombosis (Galli et al., 2003a). The report included many retrospective studies, but also a few case–control and one single cross-sectional study. As pointed out by the authors, the inherent limitations of such analysis did not allow firm conclusions to be drawn on the value of anti-β_2GPI testing. Indeed, the studies included in the analysis were not selected on the basis of their laboratory methods and no rating of the quality of the studies was done. With these limitations in mind, the results suggested that IgG anti-β_2GPI are associated with thrombosis, and this association was particularly high in SLE patients. However, among the 10 studies that included a multivariate analysis, only two found IgG anti-β_2GPI to be an independent risk factor. Several studies showed a relation between thrombosis and the presence of anti-β_2GPI, but most of these studies were performed in patient groups with a history of autoimmune disease. Another publication on the relationship between anti-β_2GPI and venous thrombosis in a general population showed an increased risk of a first episode of venous thrombosis for anti-β_2GPI-positive patients (Kaplan et al., 2004).

The role of anti-β_2GPI in arterial thrombosis remains to be established, as prospective studies investigating this relationship in a general population are lacking. Furthermore, studies from several laboratories suggest that the sensitivity of the anti-β_2GPI test for APS varies from 40% to 90% (Arvieux et al., 1992; Viard et al., 1992; Matsuura et al., 1994; Balestrieri et al., 1995; Cabiedes et al., 1995; Martinuzzo et al., 1995; Roubey et al., 1995; Katano et al., 1996; Ogasawara et al., 1996; Tsutsumi et al., 1996; Lewis et al., 1998; Galli et al., 2003a, b). Hence, the clinical value of the anti-β_2GPI ELISA as an independent risk factor for thrombosis is still uncertain.

The issue of the standardization of the anti-β_2GPI ELISA test has also been the subject of considerable debate but progress has been made in the last few years (Reber et al., 2002, 2004). There are still discrepancies among investigators with respect to important steps in the assay including: the type of plates to be used (γ-irradiated,

"untreated" vs. "high binding" type of plates); the source and purity of β_2GPI, concentration of β_2GPI used to coat the plates; and the orientation of β_2GPI coated on a surface (which appears to be related to the antibodies detected) (Iverson et al., 2002). Most importantly there is lack of consensus with respect to units of measurement for this assay. Only one study has reported on the adoption of units of measurement for the detection of these antibodies, and the establishment of ranges of positivity, cut-off levels, and intra- and inter-assay variations (Lewis et al., 1998). Furthermore, some commercial assays use bovine β_2GPI preparations, which is not the protein of choice when trying to detect anti-human β_2GPI antibodies. As a consequence, the agreement between laboratories and assays is less than satisfactory (Reber et al., 2002). In a recent study, Kaplan et al. examined the variability of anti-β_2GPI tests results in a real-world setting in which 1652 data-points of anti-β_2GPI results from 204 aPL-positive patients were examined regarding variability over time, with the testing performed in different laboratories utilizing various assays (Kaplan et al., 2004; Erkan et al., 2005). There was an agreement of 96% for initial negative to low positive anti-β_2GPI results, and 76% for moderate to high positive anti-β_2GPI results, which subsequently remained in the same range regardless of the laboratory performing the test. This study concluded that there was a moderate concordance of the results.

Another problem of the anti-β_2GPI ELISA is the frequent occurrence of low avidity anti-β_2GPI antibodies in the normal population. In an effort to improve specificity by decreasing the detection of low-affinity anti-β_2GPI antibodies, de Laat et al. (2006) performed the anti-β_2GPI ELISA using a high saline concentration, in a well-described patient cohort, to identify the risk for thromboembolism. High saline concentrations decrease the binding of low-affinity, charge-dependent anti-β_2GPI, leaving only the high-affinity anti-β_2GPI antibodies for detection. Indeed, the risk for thrombotic complications was increased in patients with salt-resistant antibodies.

Assays that utilize a phosphatidylserine, or other negatively charged phospholipids, have been proposed as providing more specific measurements of antibodies present in APS (Rote et al., 1990; Harris and Pierangeli, 1995; Merkel et al., 1999). A number of laboratories include in their test panel assays that detect antibodies directed to other negatively charged phospholipids such as phosphatidylserine, phosphatidic acid, phosphatidylinositol, or phosphatidylglycerol. Some reports have indicated that APS patients may have antibodies directed against zwitterionic phospholipids (such as phosphatidylethanolamine) or positively charged phospholipids such as phosphatidylcholine (Rote et al., 1990; Yetman and Kutteh, 1996; Sugi and McIntyre, 2001; Franklin and Kutteh, 2002). The value of these tests remains uncertain because no standardized procedure has been established and calibrators are not available.

Another potentially useful antigen for identification of aPL is the APhL phospholipid mixture prepared as a kit by Louisville APL Diagnostics Inc. (APhL® ELISA Kit). One of the authors of this chapter (SSP) was directly involved in developing both the antigen and the kit, and any evaluation of data presented in this report must be mindful of this fact. The APhL phospholipid mixture was determined on the basis of testing of aCL-positive sera from a large number of patients with or without APS. Negatively charged phospholipids were tested singly and in mixtures to determine which would best distinguish APS sera from aCL-positive non-APS samples. A mixture of phospholipids was identified that enabled such distinction, while retaining sensitivity for detection of APS. Although the APhL ELISA Kit seems to have excellent sensitivity and clinical specificity (Harris and Pierangeli, 1995; Merkel et al., 1999), this assay has not been widely used and will need to be tested in more studies by independent groups in order to confirm these findings.

2.3. The value of IgA aCL and IgA anti-β_2GPI

The value of routine IgA aCL testing in the diagnosis of APS is uncertain. Recent studies have provided data on the prevalence and significance

of IgA aCL (Wilson et al., 1998). It appears that in patients with SLE, IgA aCL are similar to IgG aCL regarding their thrombogenicity and requirement of the cofactor β_2GPI for binding to cardiolipin. In unselected patients with SLE, the prevalence of increased titers of IgA aCL has been reported to vary from 1% to 44%. The lowest reported frequency was that found by Selva-O'Callaghan et al., who detected IgA aCL in only two of their 200 patients with SLE (Selva-O'Callaghan et al., 1998). Alarcón-Segovia and colleagues, in an earlier study that included 500 patients with SLE, found increased titers of IgA aCL in 16.6% of their patients (Alarcón-Segovia et al., 1989). In another recent study, Spadaro et al. found that IgA aCL was positive in 13 (20%) of their 65 SLE patients (Spadaro et al., 2000). In contrast, Weidmann et al. found IgA aCL to be positive in 44% of 92 SLE patients and also found IgA to be the most frequent aCL isotype (Weidmann et al., 1988). The reported frequency for raised IgA aCL was even higher (52.5%) in an earlier study by Faghiri et al., where patients were preselected for being IgG or IgM aCL-positive and/or having APS-associated clinical complications (Faghiri et al., 1999). A prevalence of 83.3% was reported by López et al. in a group of patients with SLE and thrombocytopenia (López et al., 1992). As noted, the ethnic group composition of patients can influence the isotype distribution of aCL. Molina et al. studied African-American, Afro-Caribbean, and Hispanic patients with SLE and found elevated levels of IgA aCL in 16%, 21%, and 14% of these ethnic groups, respectively (Wong et al., 1991; Molina et al., 1997). The most important finding was that IgA aCL was the only aCL isotype present in 82% of aCL-positive Afro-Caribbean patients. In contrast, IgA aCL was found to be positive in only 4.4% of Chinese patients with SLE (Cucurull et al., 1999). In our experience, positive IgA aCL antibodies in the absence of IgG and/or IgM aCL are unusual in APS patients with or without SLE in the general population. In another study, Gharavi et al. found that, although IgA aCL were present in 51–55% patients with APS, most were also IgG- or IgM-positive, suggesting that measurement of IgA aCL would add little to IgG and IgM aCL determination (Gharavi et al., 1987).

Numerous studies have investigated possible associations between raised levels of IgA aPL and the clinical manifestations of the APS attributed to these autoantibodies. Several of these studies reported a significant association for IgA aCL with one or more of the main clinical manifestations of the APS. Cucurul et al., studying both aCL and anti-β_2GPI antibodies in African-American patients with SLE, found an association between thrombotic events and raised levels of both these autoantibodies (Cucurull et al., 1999). However, the number of patients with thrombotic events in their study was very small; only 5 (5%) of their 100 patients had documented evidence of thrombosis (Cucurull et al., 1999). An association between raised IgA aCL levels and thrombocytopenia in patients with SLE or other collagen vascular diseases has also been reported (Tajima et al., 1998). Finally, an association between IgA aCL and recurrent fetal loss and with unexplained spontaneous abortions has been reported in women with SLE (Kalunian et al., 1988; Bahar et al., 1993). In a recent study that tested over 700 samples from an APS registry (APSCORE), only five samples were positive for aCL IgA alone and four of these patients presented with at least one of the two major clinical manifestations of APS according to the Sapporo criteria. Studies from our own group have indicated that in animal models, IgA aCL are as thrombogenic as IgG or IgM aCL (Pierangeli et al., 1995). In a recent case series from our own unit, we found four patients with clinical manifestations of APS in which the only aPL test positive was IgA anti-β_2GPI (Pierangeli and Rand, unpublished observations).

In summary, the controversies that still exist regarding their prevalence and clinical associations are perhaps due to use of non-standardized assays and differences in study design. Because of the very low prevalence of isolated IgA APL positivity (in the absence of IgG and/or IgM aPL positivity), IgA APL testing should be recommended in cases where IgG and IgM all are negative and there is strong suspicion of APS. Despite the low prevalence of isolated IgA APL, their presence does appear to be associated with

clinical manifestations for the APS (Lee et al., 2003a, b).

2.4. Serological assays that detect antibodies to coagulation proteins in antiphospholipid syndrome

Finally, there have been an increasing number of reports from various centers using recently developed ELISA tests suggesting that patients with APS have antibodies that bind proteins other than β_2GPI. These include prothrombin (PT), protein C, protein S, and annexins A_2 and A_5 (Bevers et al., 1991; Oosting et al., 1993; Rao et al., 1995; Atsumi et al., 2000; Atsumi et al., 2004; Bertolaccini et al., 2005; de Groot et al., 2005; Cesarman-Maus et al., 2006; Galli et al., 2007).

A significant percentage of anti-PT antibodies demonstrate LAC activity (Bevers et al., 1991). Whereas LAC is a known thrombosis risk factor, currently available data are inconclusive regarding anti-PT antibodies as an independent risk factor for thrombosis (Atsumi et al., 2000; de Groot et al., 2005). However, other studies do report an increased risk for thrombosis when anti-PT antibodies are present (Forastiero et al., 2005; Bizzarro et al., 2007). Most studies only report an increased risk for thrombosis if anti-PT antibodies occur in conjunction with an established prothrombotic risk factor, such as LAC, or a systemic autoimmune disease (Hudson et al., 2003; Galli et al., 2008). The precise role of anti-PT antibodies as a marker for thrombosis thus requires further investigation. Furthermore, the detection of these antibodies appear to be influenced by the type of plates used, the presence of calcium and/or phosphatidylserine as well as other technical considerations of the assay. To date, no consensus guidelines have been established on a consistent method to perform this assay. Despite a significant number of studies published on the above-mentioned assays, the true clinical significance of these assays remains elusive and there are a number of instances where diagnosis is uncertain, since some of these tests give "false-positive" results (particularly in patients with infectious and other autoimmune diseases). To address some of these issues, we tested samples from 56 APS patients, 206 patients with infectious diseases and various autoimmune disorders, and 50 healthy controls. Samples were coded by Dr Ware Branch at the University of Utah and distributed to three other participating centers. All samples were tested in a blinded manner in the various centers utilizing: (1) an anti-β_2GPI ELISA (in-house); (2) an anti-PT ELISA (in-house); (3) an aCL (in-house) ELISA; (4) a commercial anti-β_2GPI ELISA kit (INOVA Diagnostics Inc.); and (5) a commercial aPL ELISA kit (APhL ELISA, Louisville APL Diagnostics, Inc.). The tests were considered positive when results were above the cut-off points established for each assay, for either IgG and/or IgM isotypes. Receiver operating curves (ROCs) were done to compare the areas under the curve for the various tests. Sensitivities and specificities were determined. ROCs showed that the APhL ELISA, which utilizes a mixture of phospholipids and β_2GPI instead of cardiolipin, showed the best predictive value, followed in decreasing order by the anti-β_2GPI (in-house), the aCL (in-house), the anti-β_2GPI (INOVA), and the aPT (in house) assays. Although the aPT assay showed a good specificity, the sensitivity was 24.07% and was not found positive in any APS sample in the absence of a positive aCL. APhL ELISA or anti-β_2GPI test were negative. Either aCL and/or APhL and/or anti-β_2GPI tests were found positive in all APS samples (Pierangeli and Harris, 2008). Importantly, this study validates the 2004 Sydney classification criteria for APS, with respect to the serological aPL tests recommended for the diagnosis of APS.

Antibodies directed against annexin A5 have also been shown to be present in plasma of some patients suspected of having the APS. Although not many data are available, anti-annexin A5 antibodies seem to be related to the occurrence of fetal loss (Nojima et al., 2001). Further studies are needed to clarify the role of anti-annexin A5 antibodies in APS.

In summary, determination of the value of ELISA tests utilizing protein antigens other than β_2GPI will require a thorough validation and standardization of the technical procedures involved in manufacturing and performing the

tests, and their diagnostic values remain to be established.

3. Functional assays used in the diagnosis of antiphospholipid syndrome

3.1. The lupus anticoagulant test

The LAC test measures the ability of aPL autoantibodies to prolong phospholipid-dependent clotting reactions. A positive LAC test indicates the presence of an inhibitor of coagulation that does not react directly with individual coagulation factors but rather inhibits phospholipid-dependent coagulation reactions, an effect that is not associated with bleeding complications (Triplett and Brandt, 1998). The abnormality is caused by autoantibodies, in particular those directed against β_2GPI and PT. Although aPL antibodies detected in quantitative aminoacids and LAC tests are specific for phospholipids (PLs), PL-binding proteins, or a complex of these molecules, the two tests (aCL and LAC) do not necessarily identify the same antibodies (Walton et al., 1995). With the demonstration of LAC in plasma from patients with clinical conditions other than SLE (including infections, malignancies, certain drugs, and different autoimmune disorders), and early evidence that the presence of LAC is paradoxically associated with thrombosis, it has became evident that the term LAC is in fact a misnomer (Bowie et al., 1963; Triplett and Brandt, 1988). Tests for LAC are based on the principle that aPL antibodies compete with coagulation proteins for binding sites on anionic phospholipids, thereby increasing coagulation times. Since the amount and composition of the phospholipids is a qualifying determinant in this assay, it is important to minimize the amount of residual platelets in the test plasma. As no available test for LAC is 100% sensitive, the current recommendation is to perform at least two different assays (Brandt et al., 1995a, b). Several tests are available to measure LAC, all with different sensitivities. The screening test most often used in the US and many other countries is the dilute aPTT, based on the activated partial thromboplastin time (aPTT) with lower phospholipid content to make it more sensitive for phospholipid-dependent coagulation inhibitors. However, the sensitivity of an aPTT for LAC also depends on the activator used. Tests using ellagic acid as an activator are less sensitive for LAC than tests employing other activators, such as kaolin or silica (Brandt et al., 1987, 1991; Denis-Magdelaine et al., 1995). A second popular assay to detect LAC is the dilute Russell's viper venom time (dRVVT), an assay that depends on the activation of coagulation factor X by a snake venom. The presence of LAC should always be confirmed by performing the same assay in the presence of excess phospholipids, during which the prolongation of the clotting time should be corrected. However, the absolute percentage of correction required (i.e. the ratio between the clotting time without and with excess phospholipids) to be indicative for the presence of a LAC, is still a matter of debate (Jacobsen et al., 2000).

The LAC assays have several major drawbacks. One important problem is that, based on the process of coagulation, the assay is sensitive to the presence of anticoagulant drugs, such as vitamin K antagonists and heparin. As a result, confirmation of the diagnosis of APS with this assay in patients presenting with a thrombotic event becomes difficult once the patient is treated with anticoagulant drugs.

The demonstration of LAC in patients with thrombosis has consequences for (prophylatic) treatment. To make a diagnosis of LAC, one needs an adequate test sample, sensitive screening tests, procedures to rule out deficiencies or inhibitors of coagulation factors, and procedures that show phospholipid dependency of the coagulation abnormality (Brandt et al., 1995a, b). Over the years, guidelines and criteria for the detection of LAC have been formulated and revised (Brandt et al., 1995a, b). According to the recommendation of the Scientific Standardization Committee of the International Society of Thrombosis and Haemostasis (SSC of ISTH), the following approach has been proposed: (1) screening test to evaluate if phospholipid-dependent coagulation tests are prolonged, (2) mixing test to demonstrate that the prolongation of the clotting time is caused by an

inhibitor present in plasma, and (3) confirmation of the phospholipids nature of the inhibitory antibody by adding extra phospholipids or platelet neutralization. An accurate detection of LAC is essential to facilitate the diagnosis of patients with thrombotic complications. However, the accurate identification of LAC is often difficult. No guidelines have been proposed on the nature and composition of the phospholipids used and several commercial tests for LAC detection are available. Some publications have shown that the correlation between these tests is very low and that some laboratories rely on poorly responsive screening assays. The LAC confirmatory procedures are reasonable but practically difficult, as the definitions of ''correction'' by normal plasma or by phospholipids have not been established. Unfortunately, there is no agreement on the analysis and reportage of clotting time results. It is well recognized that, along with differences in reagents, phospholipid concentrations, phospholipid composition, and instrumentation, the way clotting times are used for the calculation of LAC results is an important denominator of both the sensitivity and specificity of LAC assays. Use of fixed cut-off limits for different laboratories should be discouraged and each laboratory needs to calculate local reference ranges (Exner et al., 1990, 1991; Lupus Anticoagulant Working Party, 1990, 1991; Hemostasis Committee of the Société Française de Biologie Clinique, 1992; Working Group on Hemostasis of the Société Française de Biologie Clinique, 1993; Brandt et al., 1995a, b; Goudemand et al., 1997; Jennings et al., 1997; Arnout et al., 1999; Jennings et al., 2002; Tripodi et al., 2003).

In summary, despite considerable efforts of the standardization subcommittee on phospholipid-dependent antibodies of the SSC of ISTH for the last decade, LAC tests are still one of the most frustrating laboratory assays for routine clinical practice. Indeed, international surveys continue to show that significant percentages of predefined samples are misclassified by participating laboratories.

The LAC is less frequently positive in APS and is thus regarded as a less sensitive but more specific test for detection of aPL antibodies (Derksen et al., 1988). This specificity derives from the fact that the LAC reaction is found much less frequently in non-APS disorders. Nowadays, some consider LAC to be the most important acquired risk factor for (recurrent) thrombosis and pregnancy morbidity. Furthermore, although the majority of the patients with APS had positive aCL and LAC tests, approximately 10–16% of them are positive for LAC and negative for aCL, and 25% are positive for aCL and negative for LAC. In a recent study that utilized 107 APL-positive patients, LAC-positive with aCL-negative and anti-β_2GPI-negative was found in only 6% of the study patients (Nash et al., 2004). Antibody subsets responsible for the LAC effect overlap with, but are not necessarily the same as, the antibodies detected in the aCL assay (McNeil et al., 1989; Chamley et al., 1991; Walton et al., 1995), which may be partly due to anti-PT antibodies that, while causing a LAC effect, are not detected in the aCL assay.

The LAC assay has been shown to correlate much better with the occurrence of thromboembolic events and pregnancy morbidity than the aCL or the anti-β_2GPI assay (Galli et al., 2003b). The relation between LAC and venous thrombosis has been studied more extensively than the relation between LAC and arterial thrombosis (Galli et al., 2003b). A meta-analysis has shown that LAC activity represents a stronger risk factor for thrombosis than aCL. The analysis concluded that LAC-positive patients had an odds ratio for thrombosis 5–15 higher than controls (Galli et al., 2003a).

3.2. The predictive value of antiphospholipid antibody tests

The importance of testing for aCL antibodies in the diagnosis of APS in patients was challenged at the SSC of ISTH meeting in Boston in 2002 and more recently in a published report (Galli et al., 2008). Interestingly, the subcommittee focussed mainly on the LAC functional assay and on the solid phase assays for anti-β_2GPI. The main reason for such an approach lies with the results of a large meta-analysis study showing that LAC

is a stronger risk factor for thrombosis than the aCL assay (Galli et al., 2003a, b). However, in a subsequent study designed to validate the above proposal from the SSC of ISTH with 123 patients with persistent aPL antibodies, Nash et al. demonstrated that more than one-quarter of the cohort of patients in this report were positive for aCL and negative for LAC and anti-β_2GPI (Nash et al., 2004). This data indicate that omission of aCL testing in the evaluation of suspected APS patients, and only testing for LAC and anti-β_2GPI antibodies could have resulted in a failure to diagnose APS in a large proportion of patients, which could have led to significant changes in patient management. Furthermore, in that study, the presence of a persistent IgG aCL level of >60 GPL was shown to be a strong predictor of the presence of coexisting LAC and anti-β_2GPI (Nash et al., 2004). Subsequently, Kaplan et al. demonstrated that isolated anti-β_2GPI positivity (i.e. in the absence of aCL positivity) was observed only in 2% of the samples (Kaplan et al., 2004). The authors concluded that in aCL- and/or LAC-positive patients the anti-β_2GPI test provides little additional diagnostic value. These results suggest that perhaps anti-β_2GPI should be ordered to confirm APS (since it is more specific than the aCL test) and in situations when all other (aCL and LAC) tests are negative and there is strong suspicion for APS.

The value of multiple aPL test positivity has been addressed in only a few studies. Detkov and colleagues observed that the simultaneous presence of circulating LAC and high titers of both aCL and β_2GPI antibodies identified a subset of patients with primary APS who had a more severe clinical course of the disease. They recommend performing anti-β_2GPI in addition to LAC and aCL in order to alert the physician about the risk of a more severe course of the illness (Detkov et al., 1999). These data were then confirmed by Lee et al. (2003a, b), who demonstrated that the rate of thrombosis increased significantly from patient populations with single (27.6%) aPL test positivity to patient populations with double (38.8%) and triple (66.7%) aPL test positivity. In this study, single positivity for anti-β_2GPI accounted for 9–12% of thrombotic events and they concluded that anti-β_2GPI provided additional important information (Lee et al., 2003a, b).

The majority of the studies published so far conclude that IgG anti-β_2GPI is an important additional tool for the diagnosis of APS. Although, based on those reports, no definite conclusion can be drawn with respect to the strength of the association of thrombosis and anti-β_2GPI antibodies, it seems that anti-β_2GPI measurement may be an additional helpful test besides LAC and aCL. Its specificity seems better than aCL and it could allow the definition of APS in patients negative for LAC and aCL. To that regard, it has been shown that anti-β_2GPI may be the sole antibody present in up to 10% of the patients with clinical features of APS (Kaplan et al., 2004; Nash et al., 2004; Miyakis et al., 2006).

3.3. Annexin A5 resistance assay: a mechanistic assay for detection of pathogenic antiphospholipid antibodies

Annexin A5 (AnxA5) is a potent anticoagulant protein whose anticoagulant properties are a consequence of its high affinity for phospholipids. The protein forms a two-dimensional crystal over phospholipid bilayers and thereby blocks the availability of the phospholipids for coagulation enzyme reactions. AnxA5 is highly expressed by endothelial cells and trophoblasts, two major target cells in APS. There is direct morphologic evidence, by atomic force microscopic (AFM) imaging, that the surface-bound aPL–β_2GPI complexes can disrupt AnxA5 crystallization along with its anticoagulant activity (Rand et al., 1997).

Research in this area was initiated by the finding that the protein—which prior to the annexin nomenclature was described by several other names, including placental anticoagulant protein-I and vascular anticoagulant-α—is highly expressed in apparently a constitutive manner on the apical surfaces of syncytiotrophoblasts and that aPL antibodies reduce the quantity of AnxA5 on cultured placental trophoblasts and human vascular endothelial cells (Rand et al., 1997). It was found that the reduction of AnxA5 is due to

antibody-mediated disruption of the binding of AnxA5 to phospholipid bilayers, as demonstrated by ellipsometry, binding studies, and immunoassays using artificial phospholipid bilayers, phospholipid-coated microtiter plates, phospholipid suspension, and frozen thawed platelets (Rand et al., 1998, 2003; Hanly and Smith, 2000; Tomer, 2002; Tomer et al., 2007), and ultimately by AFM (Rand et al., 1997). The aPL antibody-mediated disruption of Anx5 binding has been confirmed by several groups (Hanly and Smith, 2000; Tomer, 2002; Cederholm et al., 2005; Tomer et al., 2007; Gaspersic et al., 2007). However, one laboratory reported that it was unable to confirm this effect by ellipsometry (Willems et al., 2000).

These observations were translated by Rand and colleagues into assays of patient blood samples for AnxA5 displacement and for resistance to AnxA5 anticoagulant activity (Rand et al., 1998, 2004) (the principle underlying these assays is described in Figs. 1 and 2). The assays are based on the concept that several critical coagulation reactions require phospholipids for the assembly of coagulation enzyme–cofactor–substrate complexes, and these reactions can occur unimpeded in the absence of AnxA5. However, when AnxA5 is present in solution, the protein rapidly binds to the phospholipids and forms two-dimensional crystals over them that impede coagulation proteins from binding and forming enzyme–cofactor–substrate complexes. APL antibodies are able to disrupt the ordered binding of AnxA5, creating defects in the crystalline array that result in a net increase in the availability of phospholipid. This interference with the anticoagulant activity of AnxA5 thereby accelerates coagulation reactions. The antibody-mediated interference with AnxA5 binding can be monitored in two ways: (1) by measuring the effects upon AnxA5 anticoagulant activity (i.e. acceleration of coagulation times), and (2) by measuring the effects on the binding of a labeled AnxA5 to phospholipids. Rand and colleagues' clinical

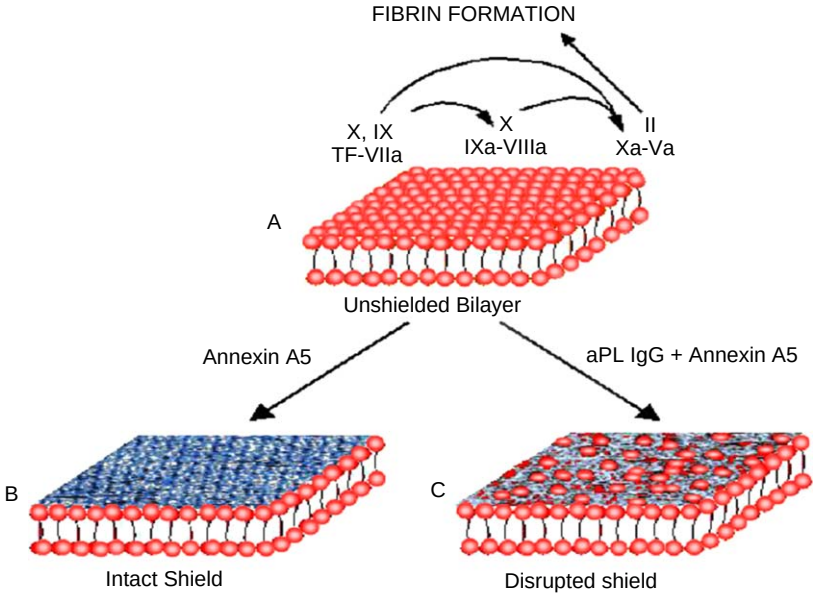

Figure 1. Model for antiphospholipid antibody (aPL)-mediated disruption of the annexin A5 (AnxA5) shield on placental trophoblasts. (a) Apical membranes of placental trophoblasts, if unshielded by AnxA5, expose thrombogenic phospholipids (red polar heads)—particularly phospholipids that accelerate the critical phospholipid-dependent coagulation reactions shown and promote fibrin formation in the intervillous space. (b) AnxA5, expressed by trophoblasts in an apparently constitutive manner, forms two-dimensional crystals (gray array, adapted from an AFM image) over the phospholipids that shield them from binding coagulation protein complexes. (c) aPL antibodies and cofactors (mainly β_2GPI) create defects in the AnxA5 crystalline arrays that expose the phospholipids and accelerate coagulation reactions. (See Colour Plate Section.)

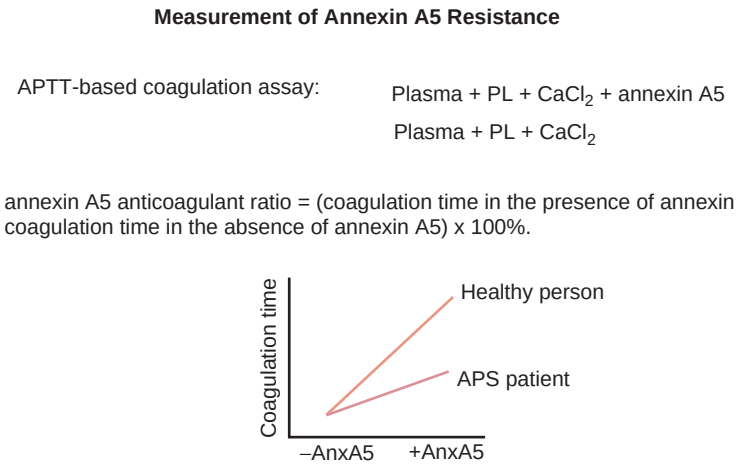

Figure 2. Principle of the annexin A5 (AnxA5) resistance assay. In the first phase of this assay, the tissue factor–phospholipid reagent is incubated with the test plasma in the absence of calcium. This allows aPL antibodies, if present, to bind to the phospholipids. The phospholipid sample is then washed free of the plasma and split into two equal aliquots. Both are added to normal pooled plasma and coagulation is triggered by the addition of calcium plus the anticoagulant AnxA5 to one aliquot, and the addition of calcium alone to the other. The coagulation times of the two samples, with and without AnxA5, are compared and the results are expressed as an "anticoagulant ratio." The reduction of the AnxA5 anticoagulant ratio is described as "AnxA5 resistance." aPTT, activated partial thromboplastin time.

studies have indicated that the functional anticoagulant assay is simpler to perform and appears to correlate better with clinical manifestations of the APS disease process than the assay for antibody-mediated displacement of AnxA5 (Rand et al., 2004; Wu et al., 2006a, b; de Laat et al., 2007).

The assay for detection of resistance to AnxA5 anticoagulant activity is based upon the classical PT and aPTT assays. The methodology is described in Rand et al. (2004) and further refined in de Laat et al. (2007). Briefly, samples are incubated with a phospholipid suspension that includes tissue factor (TF) as the test substrate, and the test sample-treated phospholipid is added to normal plasma in the presence and absence of AnxA5, which is then recalcified with calcium. The coagulation times in the presence and absence of AnxA5 are measured, and the results are expressed as the "AnxA5 anticoagulant ratio" (Fig. 2). Using the AnxA5 resistance assay, Rand and colleagues showed that patients with aPL antibodies and thrombosis and/or with pregnancy losses had significantly reduced AnxA5 anticoagulant activity. This assay not only confirms a novel mechanism for thrombosis in APS but also enables the identification of another subset of aPL antibodies from patients with APS and clinical manifestations. Hence the AnxA5 resistance assay is an excellent candidate for a mechanistic assay for APS since it has biologic plausibility, there is quantitative and atomic force imaging evidence for its occurrence, and there is strong evidence for in vivo relevance in humans. This assay was patented by Dr Rand and assigned to the Mount Sinai School of Medicine (United States Patents 6284475 and 7252959—Assays for diagnosis of thrombophilic disease).

4. Conclusions and future perspectives in testing for antiphospholipid antibodies

There is no doubt that the aCL, the anti-β_2GPI, and the LAC are useful tests for the diagnosis of APS. However, limitations of these assays have caused uncertainty and misinterpretation of their value. Utilization of validated ELISA assays, whether commercial or in-house, with well-tested calibrators may enable more reproducible measurements.

Attempts to standardize the above-mentioned tests used to confirm diagnosis of APS have been only partially successful. One possible way to address this problem would be through an international effort, that should include centers from all continents, to reach consensus and approve guidelines to run the aPL ELISAs and LAC tests. This would also have to be based not only on personal experience but on the thorough and rigorous examination of scientific evidence accumulated over the years.

The currently available assays for detection of aPL antibodies identify patient subgroups at risk for thrombosis. At the moment, the best available option to detect patients at risk for thrombotic events appears to be a combination of positive test results, in particular the combination of a positive aCL ELISA, LAC assay and anti-β_2GPI antibodies ELISA. In fact, evidence is accumulating that patients testing positive for more than one single aPL assay have an increased risk of thrombosis or pregnancy morbidity. Although a combined positive tests result in all of these three assays seems to identify patients at high risk for thrombosis, it remains a matter of debate whether determining antibody profiles is indicated in every patient presenting with thrombosis or pregnancy morbidity, including those without a history of autoimmunity.

New assays, such as an ELISA that specifically detects antibodies directed against domain I (the immunodominant epitope recognized by anti-β_2GPI antibodies) and the AnxA5 resistance assay appear to be very promising. Results from multicenter studies investigating the correlation between these antibodies and clinical manifestations are awaited with interest. However, it is uncertain whether these more specific assays will pick up all pathological antibodies, because we do not know whether only one subpopulation of aPL is responsible for all the clinical manifestations of APS or whether there are different pathological subpopulations of aPL with different clinical associations. We expect that increased knowledge of the pathophysiology of the APS will ultimately provide definitive answers to these questions.

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CHAPTER 4

Mechanisms of Action of Antiphospholipid Antibodies

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1. Introduction

The antiphospholipid syndrome (APS) is a condition characterized by recurrent arterial/venous thrombosis and/or fetal losses associated with the persistent presence of antiphospholipid antibodies (aPL) detectable by functional coagulation test (lupus anticoagulant—LAC) and/or solid phase assays (anticardiolipin (aCL) and anti-β_2 glycoprotein I (anti-β_2GPI)) (Miyakis et al., 2006).

The close association between the presence of aPL and the clinical manifestations of the syndrome in several epidemiological studies as well as the description of in vitro and in vivo experimental models strongly support the concept that APS is an autoantibody-mediated disease. In this regard, aPL represent not only formal diagnostic markers but also pathogenic autoantibodies (De Laat et al., 2008).

aPL may trigger clotting by several mechanisms. Such a heterogeneity fits well with the fact that thrombosis may be sustained by events that can be different depending on the type of vessel affected (arterial vs. venous vessels, for example). Moreover, thrombosis itself cannot account for all the clinical manifestations of APS. In fact, thrombotic events do not appear to represent the main pathogenic event in the defective placentation responsible for aPL-associated fetal losses or APS nephropathy (Amigo, 2006; Meroni et al., 2008). Accordingly the present chapter will cover in separated sections the main aPL-mediated mechanisms for thrombosis as well as those responsible for other aPL-related effects.

aPL are now known to react with phospholipid-binding proteins rather than with phospholipid alone, β_2GPI being the most important target autoantigen (De Laat et al., 2008). β_2GPI is one of the most abundant human plasma proteins. It is produced in large quantities by liver cells and by placenta, but its true physiological function is still matter of debate. β_2GPI interacts with several coagulation components and with different cell types (Giannakopoulos et al., 2007; Meroni, 2008). The autoantibody binding to the protein complexed with the coagulation factors or expressed on the cell membranes may induce different biological effects that have been shown to contribute to the whole spectrum of the clinical manifestations of APS. More controversial is the role of the other major phospholipid-binding protein recognized by aPL: prothrombin (PT) (Giannakopoulos et al., 2007). In addition, a specific section of this chapter will cover the new molecular information about the interaction between β_2GPI and its candidate cell membrane receptors or coagulation components.

The recently updated APS classification criteria state that "for histopathologic confirmation,

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thrombosis should be present without significant evidence of inflammation in the vessel wall" (Miyakis et al., 2006). Accordingly APS is classically accepted to represent a non-inflammatory vasculopathy. However, there is emerging evidence that aPL may trigger inflammatory mediators, as shown in experimental models (Cavazzana et al., 2007).

2. Thrombotic events mediated by antiphospholipid antibodies

There is strong evidence that aPL can mediate clotting in in vivo animal models (Jankowski et al., 2003; Fischetti et al., 2005; Vega-Ostertag and Pierangeli, 2007). It is widely accepted that aPL react mainly with phospholipid-binding proteins such as β_2GPI and prothrombin. Because of the interaction of these two proteins with several clotting factors and with cells involved in the coagulation homeostasis, it is not surprising that different pathogenic mechanisms have been reported to explain the aPL thrombogenic activity. However the evidence is stronger for a pathogenic role of antibodies directed against β_2GPI compared with antibodies against PT.

A "two-hit hypothesis" has been suggested to explain the clinical observation that thrombosis occurs only occasionally in spite of the persistent presence of aPL. This states that aPL (first hit) increase the thrombophilic risk and the clotting takes place in the presence of another thrombophilic condition (second hit) (Shoenfeld et al., 2006). It has been suggested that inflammatory responses may be the "second hit," in particular those related to infections since they frequently precede the full-blown syndrome and may be the initiators of the subtype known as catastrophic APS (Shoenfeld et al., 2006).

As shown in Fig. 1, aPL have been reported to interfere with: (1) natural anticoagulant mechanisms, (2) fibrinolysis, (3) platelets, (4) endothelial cells, and (5) monocytes. All these effects may cooperate in increasing the thrombophilic state of patients and in triggering clotting when a second hit does occur.

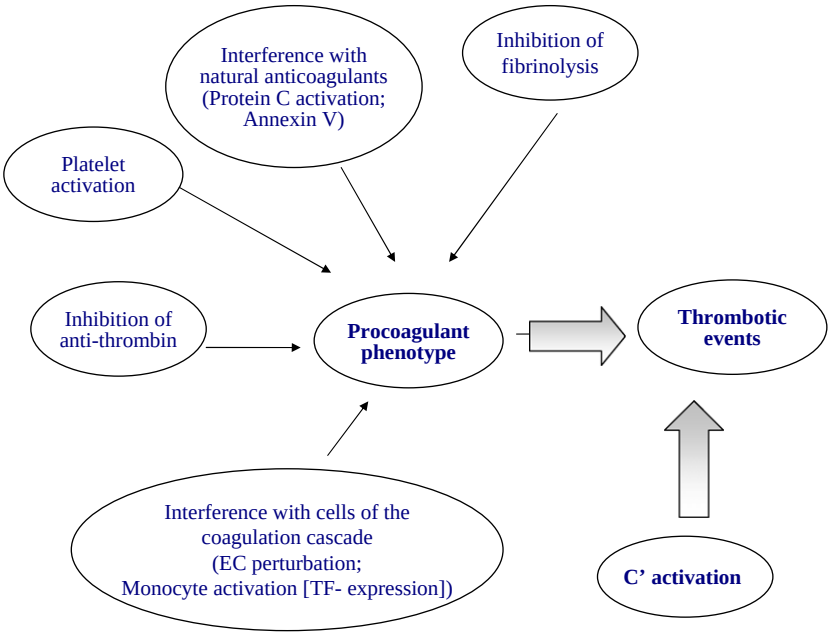

Figure 1. Antiphospholipid antibody pathogenic mechanisms mediating thrombosis.

2.1. Interference of antiphospholipid antibodies with anticoagulant systems

2.1.1. Activated protein C

Activated protein C (APC) displays an anticoagulant role by binding and inactivating the procoagulant factors Va (FVa) and VIIIa. β_2GPI/anti-β_2GPI antibody complexes may interfere with the activated protein C assembling/function, eventually favoring a procoagulant phenotype. APL with specificity for PT (anti-PT, aPT) may also affect activated protein C function. In addition, some human monoclonal antibodies derived from APS patients were reported to react with activated protein C and able to inhibit its anticoagulant function (reviewed in Giannakopoulos et al., 2007).

2.1.2. Antithrombin III

A small percentage of aCL-positive sera cross-reacts with glycosaminoglycans such as heparin and heparan sulfate. An aCL-positive serum able to inhibit the heparin-dependent activation of antithrombin III by up to 80% has been reported (Chamley et al., 1993).

2.1.3. Annexin A5

Annexin A5 is a cationic protein that binds anionic phospholipid with high affinity, and forms a protective anticoagulant shield. β_2GPI/anti-β_2GPI antibody complexes (in particular antibodies directed against β_2GPI domain I) may disrupt such a shield, favoring thrombosis and fetal loss (De Laat et al., 2007). Although a reduced annexin A5 deposition on placenta villi of APS patients with fetal loss was reported, the true pathogenic role for such a mechanism is still debated since annexin A5 deficiency in knockout mice does not have an impact on litter size and fetus viability (Rand et al., 1994; Brachvogel et al., 2003).

2.2. Interference with fibrinolysis

Polyclonal as well as human monoclonal IgG aPL can impair fibrin dissolution by plasmin and fibrinolysis on endothelial cells in vitro (reviewed in Kolev et al., 2002; Giannakopoulos et al., 2007). Anti-tissue plasminogen activator (tPA) antibodies correlated with reduced tPA plasma levels have also been described in a few patients (reviewed in Giannakopoulos et al., 2007). Accordingly, some human monoclonal antibodies from APS patients react with and inhibit tPA (Lu et al., 2005). In addition, antibodies against annexin A2 receptor have been demonstrated in APS patients. These antibodies may interfere with the generation of plasmin by affecting the interaction between tPA, plasminogen, and annexin A2 receptor on endothelial cells (reviewed in Giannakopoulos et al., 2007). All these mechanisms may contribute to the impairment of fibrinolysis in APS patients. Certain human monoclonal antibodies may react with different members of the serine protease (SP) family, such as thrombin, activated protein C, plasmin, tPA, and procoagulant factors (FVIIa, FIXa and FXa) (reviewed in Hwang et al., 2001; Lu et al., 2005; Giannakopoulos et al., 2007). Cross-inhibition studies apparently support the hypothesis that these antibodies cross-react with β_2GPI and enzymatic domains in SP molecules (Lu et al., 2005; Lin et al., 2007).

2.3. Interference with cells of the coagulation cascade

2.3.1. Endothelial cells

Several groups have reported anti-endothelial binding activity in sera from both primary and secondary APS patients. Such a reactivity was mainly related to the ability of aPL to recognize β_2GPI expressed on the cell membranes, although antibodies directly reacting with other constitutive endothelial membrane proteins have also been found (reviewed in Meroni et al., 2004b).

In vitro studies carried out by different groups have shown that aPL (mainly-β_2GPI dependent) cause endothelial cell perturbation, inducing a procoagulant and proinflammatory phenotype (reviewed in Meroni et al., 2004b).

The procoagulant phenotype is supported by the demonstration of: (1) tissue factor (TF) expression upregulation, (2) induction of endothelial apoptosis, (3) downregulation of anticoagulant mechanisms

such as the reduction of prostacyclin secretion, protein C/S activation, and competition for annexin A5 binding. Increased expression of mRNA for pre-proendothelin 1 has also been reported and suggested to play a role in arterial thrombosis (reviewed in Meroni et al., 2004b).

The aPL-induced endothelial proinflammatory response has been extensively characterized as adhesion molecule upregulation and proinflammatory cytokine and chemokine synthesis and secretion (reviewed in Meroni et al., 2004b; Hamid et al., 2007).

aPL-mediated endothelial cell perturbation was found to be mediated through nuclear factor-κB (NF-κB) translocation and p38 mitogen-activated protein kinase (MAPK) activation (Meroni et al., 2004b).

Endothelial cell perturbation has also been reproduced in in vivo experimental models and shown to be correlated with enhanced thrombus formation (Vega-Ostertag and Pierangeli, 2007). It is still debated whether or not indirect markers of endothelial perturbation can be found in APS patients (Meroni et al., 2004a).

2.3.2. Platelets

A potential aPL binding to platelets followed by their aggregation has been suggested by the frequent low platelet count in APS patients as well as by the thrombocytopenia found in APS animal models (Blank et al., 1991; Miyakis et al., 2006). Accordingly, elevated levels of platelet-derived thromboxane metabolic breakdown products were reported in the urine of APS patients and a correlation with aPL titers was found (Reverter and Tàssies, 2006). Upregulation of platelet markers of activation (CD62, CD63 cell surface expression, annexin A5 and PAC-1 binding, β-thromboglobulin secretion, release of soluble CD62, of platelet-derived microparticles, increased leukocyte-platelet complexes) were shown in some studies but not confirmed in other reports (Reverter and Tàssies, 2006). Nevertheless, these findings are suggestive for an in vivo platelet activation in APS. Accordingly, the in vivo infusion of an anti-β_2GPI monoclonal antibody into hamsters whose carotid arteries were primed with a photochemical injury induced platelet-rich thrombi (Jankowski et al., 2003).

Moreover, the ability of aPL to activate platelets in vivo was also supported by recent unpublished observations by Pierangeli and colleagues who showed that: (1) thrombus formation was not increased by aPL in GPIIb/IIIa-deficient (β_3 null) mice and (2) aPL-mediated thrombus formation in vivo was reduced by pretreatment of the mice with a monoclonal anti-GPIIb/IIIa antibody (1B5) (Pierangeli et al., unpublished observations).

aPL bind activated but not resting platelets and the binding is strictly β_2GPI-dependent (Reverter and Tàssies, 2006). Once bound, aPL may: (1) increase platelet aggregation/activation in the presence of low concentrations of thrombin, ADP, or collagen, compared with IgG aCL from syphilis patients and IgG from healthy subjects (Reverter and Tàssies, 2006), (2) increase platelet thromboxane production in the presence of low doses of thrombin, and (3) enhance the expression of platelet membrane glycoproteins, particularly GPIIb/IIIa and GPIIIa, when platelets are pretreated with suboptimal doses of a thrombin receptor agonist peptide (TRAP). These effects can be abrogated by pretreatment of the platelets with hydroxychloriquine (Espinola et al., 2002).

The need for platelet submaximal activation by different agonists (thrombin, collagen, etc.) was thought to be responsible for phosphatidylserine exposure on the cell outer membrane, eventually favoring β_2GPI adhesion and reactivity with β_2GPI-dependent aPL (Lutters et al., 2003).

aPL activate platelets and induce thromboxane B_2 (TXB_2) production, mainly through the activation of p38MAPK and subsequent phosphorylation of cytosolic phospholipase A_2 ($cPLA_2$) (Lutters et al., 2003; Vega-Ostertag and Pierangeli, 2007). IgG aPL may also interact with the receptor for the Fc fragment of the IgG molecule (FcγR); however there is no evidence for a major role for such a mechanism in platelet activation (reviewed in Reverter and Tàssies, 2006). Recently, anti-β_2GPI antibody–β_2GPI complex has been reported to induce inappropriate platelet activation via the glycoprotein Ibα (GPIbα) receptor (Shi et al., 2006).

2.3.3. Monocytes

Tissue factor upregulation has been advocated as an important mechanism responsible for the aPL

prothrombotic effect. Besides endothelial cells, upregulation of TF expression and function has also been reported in monocytes (reviewed in López-Pedrera et al., 2006). Such an upregulation was found when normal cells were incubated in vitro with aPL (in particular with β_2GPI activity) as well as in peripheral blood monocytes from APS patients (reviewed in Vega-Ostertag and Pierangeli, 2007).

Antiphospholipid antibody-induced monocyte activation and TF upregulation is mediated via NF-κB translocation and p38MAPK activation (reviewed in Vega-Ostertag and Pierangeli, 2007). Vascular endothelial growth factor (VEGF) may stimulate TF expression in monocytes through its receptor tyrosine kinase Flt-1. Indeed a recent study has shown that VEGF and Flt-1 expression are increased in the monocytes of APS patients (Cuadrado et al., 2006).

Altogether, these findings strongly support the suggestion that aPL may induce a monocyte procoagulant phenotype both in vitro and in vivo.

3. Antiphospholipid-mediated mechanisms responsible for fetal loss

Intraplacental thrombosis with maternal–fetal blood exchange impairment was initially thought to be the main pathogenic mechanism underlying APS-associated obstetric manifestations. However, histological analysis of placentas and abortive material from women with APS has shown not only vascular thrombosis but also acute and chronic inflammatory signs, suggesting that more than one mechanism is involved (Fig. 2) (reviewed in Di Simone et al., 2007).

3.1. Thrombotic manifestations

Experimental models have supported the role of thrombotic events as one of the pathogenic mechanisms in aPL-mediated fetal loss. In particular histological studies of placentas from women with APS show widespread thrombosis and infarction both in first and second trimester abortions (reviewed in Di Simone et al., 2007). In addition, in vitro studies have reported that aPL are able to induce a procoagulant phenotype at the placental level with a significant increase in thromboxane synthesis (reviewed in Di Simone et al., 2007) and to interfere with the anticoagulant activity of the annexin A5 shield on the trophoblast (Rand et al., 1994; Di Simone et al., 2007).

However, intravascular or intervillous blood clots are rarely found on histological examination of miscarriage samples from patients with APS and histopathological findings suggestive of thrombosis cannot be found in the majority of the placentas from women with APS (reviewed in Di Simone et al., 2007). In addition, there is no strong evidence that thrombosis plays a role in early pregnancy losses.

3.2. Defective placentation

It has been suggested that aPL may induce defective placentation by interfering with early trophoblast invasion without necessarily triggering thrombosis (reviewed in Di Simone et al., 2007). Accordingly, there is evidence for a direct aPL effect on both maternal decidua and invading trophoblasts.

Experimental in vitro studies show that aPL (in particular β_2GPI-dependent antibodies) might directly bind human trophoblast, impairing its differentiation/maturation. The antibodies may in fact induce: (a) a direct cellular injury, (b) apoptosis, (c) inhibition of proliferation and syncytia formation, (d) decreased human chorionic gonadotrophin (hCG) production, (e) defective invasiveness, and (f) expression of integrins and cadherins, eventually interfering with decidual invasion (reviewed in Di Simone et al., 2007).

Both murine and human monoclonal as well as polyclonal IgG antibodies from APS patients have been found to bind trophoblast monolayers in vitro (reviewed in Di Simone et al., 2007). In some studies authors have specifically demonstrated that the antibody binding was dependent on the expression of β_2GPI on the trophoblast cell membranes (reviewed in Di Simone et al., 2007). Being a cationic protein, β_2GPI might bind to phosphatidylserine exposed on the external cell membranes of

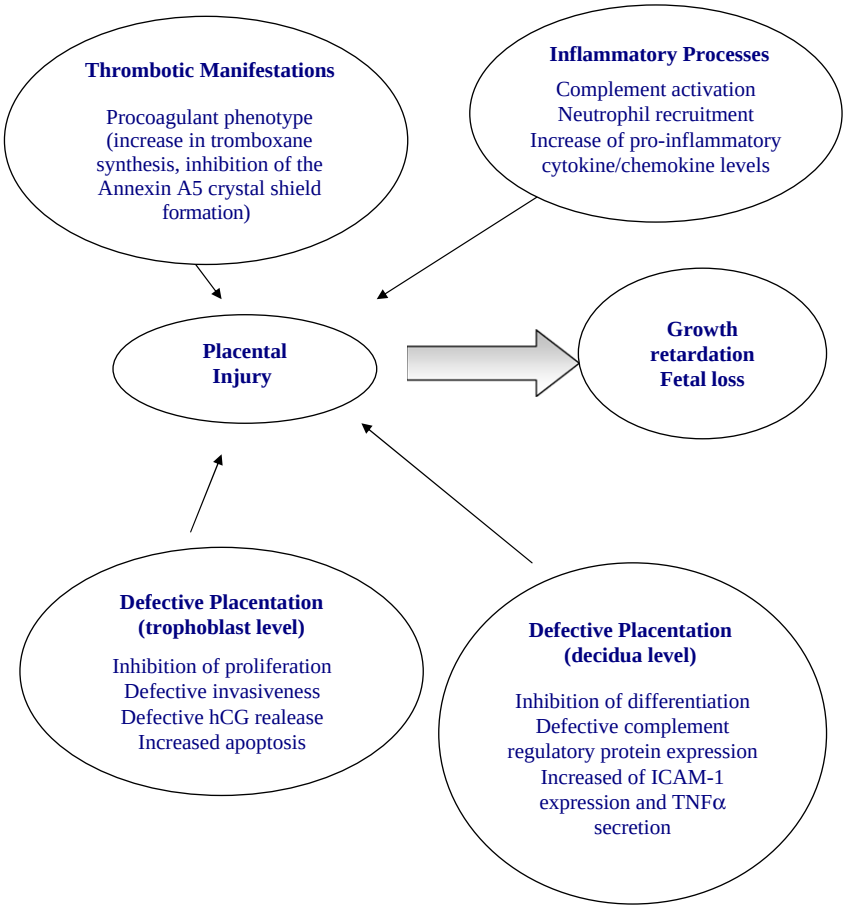

Figure 2. Antiphospholipid antibody pathogenic mechanisms mediating fetal losses.

trophoblasts undergoing syncitium formation. Such an expression is a prerequisite to an explanation of aPL/anti-β_2GPI antibody placental tropism and explains why aPL passively infused in naïve pregnant mice rapidly disappear from the circulation and are entrapped in placenta tissues (reviewed in Di Simone et al., 2007).

In addition to the effects on the trophoblast, aPL may also affect the maternal side of the placenta. In fact, impaired endometrial differentiation and lower expression of complement regulatory proteins (DAF/CD55) was found on endometrial biopsies, suggesting that these abnormalities before conception may compromise implantation and predispose to complement-mediated pregnancy failure (Francis et al., 2006). We found that human β_2GPI-dependent aPL (both polyclonal and monoclonal) react with human stromal decidual cells in vitro and induce a proinflammatory phenotype (e.g. increased ICAM-1 expression and tumor necrosis factor alpha (TNF-α) secretion) (Borghi et al., 2007).

The role of β_2GPI expressed on the decidual cell membranes as the antigenic target was further supported by the fact that comparable data could be reproduced using affinity purified anti-β_2GPI polyclonal IgG fractions (Borghi et al., 2007).

In conclusion, aPL-associated fetal loss may be caused by a defective placentation process sustained by different non-thrombotic pathogenic mechanisms affecting both the fetal and the maternal placental tissues.

4. Antiphospholipid-associated inflammatory events

During a physiological pregnancy a fine balance between pro- and anti-inflammatory mediators with differences depending on the time of gestation may occur. It is widely accepted that acute inflammatory events are generally responsible for a negative pregnancy outcome (Chaouat, 2007). Accordingly, soluble proinflammatory mediators have been shown to play a role in experimental animal models of aPL-induced fetal loss. This is the case for complement, proinflammatory cytokines (TNF-α), and CC chemokines.

In a similar way, inflammation may favor a procoagulant state and vice versa (Danese et al., 2007). In line with this coagulation–inflammation circle, the in vivo thrombogenic effect of aPL has been shown to be dependent on complement activation in two different animal models of aPL-induced thrombosis (Fischetti et al., 2005; Pierangeli et al., 2005; Romay-Penabad et al., 2007).

4.1. Antiphospholipids induce fetal loss by inflammatory mediators

Repeated intraperitoneal injection of large amounts of human IgG with aPL activity (10 mg/mouse) to pregnant naïve mice when implantation has already taken place induces a strong placental inflammatory damage that results in resorption and fetal growth retardation evaluated at day 15 of pregnancy. In this in vivo model, immunohistological examination of the decidua showed: (1) human IgG and murine complement deposition, and (2) local acute inflammation characterized by neutrophil recruitment and local as well as systemic TNF-α secretion (Holers et al., 2002; Girardi et al., 2003; Berman et al., 2005). Recent findings point out that an upregulated TF expression on infiltrating neutrophils may also play a role (Redecha et al., 2007).

Inhibition of complement cascade activation using the C3 convertase inhibitor complement receptor 1-related gene/protein y (Crry)-Ig protects from fetal loss, growth retardation, and immunohistological lesions (Holers et al., 2002). Accordingly, the same experimental model carried out in mice deficient in complement C3 showed resistance to the aPL-mediated fetal injury (Holers et al., 2002). Additional studies demonstrated that the complement component C5 (and particularly its cleavage product C5a) plays a key role in the model (Girardi et al., 2003). Authors have suggested that a complement-dependent placental inflammation may be responsible for the aPL-mediated fetal loss. In line with this hypothesis the same group demonstrated that the protective effect of heparin in this model was closely linked to its anticomplementary activity rather than to its anticoagulant activity (Berman et al., 2005).

The intravenous injection of much smaller amounts of human IgG with aPL activity (10–50 μg/mouse) before the implantation takes place, was reported by other groups to induce resorptions and fetal growth retardation as well (Blank et al., 1991; Martinez de la Torre et al., 2007). This model does not apparently require huge inflammatory (and complement deposition) events at the placental levels (Nebuloni et al., 2009, in press). Nevertheless, we have recently demonstrated that proinflammatory chemokines are involved in this experimental model (Martinez de la Torre et al., 2007). In fact, mice deficient for D6—a placental receptor which recognizes and targets to degradation most inflammatory CC chemokines—are more susceptible to fetal loss when passively infused with human aPL IgG than pregnant wild-type mice. In other words, D6 deficiency enables a chemokine overproduction, leading to more inflammation and eventually to pregnancy loss.

There is no conclusive evidence for complement deposition in human abortive material or in placentas from women with APS up to now. In fact, one retrospective study reported an excessive complement product deposition in placentas (particularly at the trophoblast level) of viable term infants from patients with aPL (Shamonki et al., 2007). However, another prospective study on abortive specimens obtained from a small series of aPL-related fetal deaths was unable to show any complement deposition (Cavazzana et al., 2007). The same group, however, recently reported complement deposition in a larger series of APS abortive specimens and at-term placentas (Tedesco, 2008).

4.2. Antiphospholipid thrombogenic effect is complement mediated

Mice deficient in complement components C3 and C5 were found to be resistant to the enhanced thrombosis and endothelial cells activation that was induced by the passive infusion of human aPL IgG. Furthermore, inhibition of C5 activation using anti-C5 monoclonal antibodies prevented thrombophilia in this animal model (Pierangeli et al., 2005). The same group confirmed their previous results by showing that C5a receptor-deficient mice are protected from the thrombogenic effects of some aPL (Romay-Penabad et al., 2007). Using a different animal model, Fischetti et al. (2005) demonstrated that β_2GPI-dependent aPL IgG fractions from APS patients are able to trigger clotting in the presence of a submaximal LPS injection in the rat mesenteric microcirculation. The number of platelet–leukocyte aggregates and thrombotic occlusions was markedly reduced in C6-deficient rats and in animals treated with anti-C5 minibody, suggesting the contribution of the terminal complement complex to the aPL IgG-mediated intravascular thrombosis (Fischetti et al., 2005). The ability of aPL to fix complement was also confirmed by showing that the same IgG fractions were able to fix C1 q in vitro (Fischetti et al., 2005).

5. Antiphospholipids recognize β_2GPI expressed on the surface membranes of different cell types

Several APS pathogenic mechanisms are mediated by aPL reacting with β_2GPI expressed on the surface membranes of different cell types involved in the coagulation cascade, in placentation, as well as in tissues that are known targets for aPL-mediated damage. Notwithstanding the nature of the receptors for β_2GPI, cell membrane binding is still matter of investigation.

In this section we will discuss the interaction of β_2GPI with different cell types (Table 1) and the well-known receptors involved in the pathogenesis of APS.

Table 1
Interaction of β_2 glycoprotein I with different cell types

Cell types	Candidate receptors
Cells involved in coagulation	
Endothelial cells	Heparan sulfate, annexin A2, TLR4, ApoER2′
Monocytes	Annexin A2, TLR4
Platelets	GPIbα, ApoER2′
Cells of placental tissues	
Trophoblast	Annexin A2, TLR2, TLR4
Stromal decidual cells	TLR4, annexin A2, ApoER2′
Other cell types	
Renal epithelial cells	Megalin
Fibroblast	TLR2

5.1. Cells involved in coagulation

5.1.1. β_2 Glycoprotein I endothelial cells receptors

There is a large body of evidence from in vitro studies and some in vivo experimental models that β_2GPI binds to endothelial cells membrane, offering suitable epitopes for aPL binding (Fischetti et al., 2005; Meroni, 2008).

Once bound, aPL may perturb endothelial cells through NF-κB and p38 MAPK pathways. However the nature of the receptor(s) for β_2GPI and the signaling cascade triggered are only partially known (Raschi et al., 2003; Vega-Ostertag and Pierangeli, 2007).

Studies with mutants of the molecule suggested that β_2GPI binds endothelial cells through the electrostatic interaction between the cationic phospholipid-binding site of domain V and, at least in part, the anionic heparan sulfate exposed on endothelial cells membranes. However, enzymatic digestion of endothelial heparan sulfate only partially inhibits the β_2GPI endothelial binding, suggesting that additional structures might also be responsible for β_2GPI binding (reviewed in Meroni et al., 2004b).

In this regard, annexin A2 and toll-like receptor 4 (TLR4) have recently been suggested as candidate receptors (Raschi et al., 2003; Zhang and McCrae, 2005). Annexin A2 is a receptor for tissue plasminogen activator (tPA) and plasminogen, it is expressed on endothelial cells as a membrane protein without

an intracellular tail. Although experiments with cells transfected with human annexin A2 clearly showed that it behaves as a receptor for β_2GPI, the presence of an "adaptor" protein (or a co-receptor) is required for the transduction of the signal and the eventual cell activation (Zhang and McCrae, 2005).

We found that the in vitro intracellular signaling induced by anti-β_2GPI antibodies on endothelial cells is mediated by the myeloid differentiation factor 88 (MyD88)-dependent pathway (Raschi et al., 2003). In line with this finding, lipopolysaccharide non-responsive ($LPS^{-/-}$) mice displaying a single point mutation on the *tlr4* gene are protected against the thrombogenic effect of passively infused aPL. Moreover, a reduced prevalence of protective *tlr4* gene polymorphisms was reported in APS patients with previous thrombotic events compared with that in the normal population (Pierangeli et al., 2007).

As a whole, these data suggest the involvement of TLR4 in anti-β_2GPI antibody-mediated effects in vivo. Furthermore, preliminary data apparently suggest that TLR4 may bind immobilized β_2GPI in Affi-Gel HZ, indicating that TLR4 may behave as the co-receptor for annexin A2 (Zhang et al., 2004).

Nevertheless, a definite demonstration of the direct binding of β_2GPI to TLR4 is still a matter of research.

Apolipoprotein E receptor 2 (ApoER2′) is a receptor belonging to the low-density lipoprotein (LDL) family and is expressed on several cell types. It has been found to be one of the platelet receptors for β_2GPI described on platelets and involved in aPL activation (Lutters et al., 2003). ApoER2′ is also expressed on endothelial cell membranes; preliminary experiments carried out on endothelial cell monolayers showed that an anti-ApoER2′-blocking antibody may be able to partially inhibit aPL (β_2GPI-dependent) binding and endothelial cell activation (Raschi et al., 2009, in press).

As a whole, these findings suggest that more than one endothelial cell membrane structure is involved in binding of β_2GPI.

5.1.2. β_2 Glycoprotein I monocyte receptors

Sorice et al. (2007) have recently identified β_2GPI and its putative receptor annexin A2 in lipid raft fractions of human monocytes. Moreover, there was an association between β_2GPI and TLR4, suggesting that it was partially dependent on raft integrity. Monocyte activation through IL-1 receptor-associated kinase (IRAK) phosphorylation and NF-κB translocation was hypothesized to be dependent on the interaction between β_2GPI/anti-β_2GPI antibody complexes and both annexin A2 and TLR4 (as co-receptor) (Sorice et al., 2007).

5.1.3. β_2 Glycoprotein I platelet receptors

Anti-β_2GPI antibodies aggregate platelets in the presence of β_2GPI by crosslinking both the GPIbα receptor (Shi et al., 2006) and ApoER2′ (Reverter and Tàssies, 2006).

The GPIbα is a subunit of the GPIb-IX-V platelet receptor and von Willebrand factor (VWF) is its most important ligand (Shi et al., 2006). The interaction of GPIbα with β_2GPI has been demonstrated under flow conditions that immobilized and dimerized β_2GPI triggers platelet adhesion in a GPIbα-dependent manner (Lutters et al., 2003). The mechanism proposed for platelet activation by anti-β_2GPI antibodies involves the induction of the phosphoinositide-3 kinase/Akt and p38 MAPK/phospholipase A_2 pathways, which end in TXA_2 production (Lutters et al., 2003).

Recent findings have shown that β_2GPI is a biological inhibitor of VWF, interfering with VWF-dependent platelet adhesion. The authors also demonstrated that anti-β_2GPI antibodies may neutralize β_2GPI–VWF interactions and thus the inhibitory effect of β_2GPI. This mechanism may contribute to the thrombophilic state in APS, although the lack of thrombotic events in subjects deficient of β_2GPI as well as in $\beta_2GPI^{-/-}$ mice do not support a main role for such a mechanism (Hulstein et al., 2007).

Lutters et al. (2003) showed that the increased platelet adhesion to collagen induced by the β_2GPI/anti-β_2GPI complex is lost when platelet ApoER2′ is blocked using receptor-associated protein (RAP). As further evidence of the direct interaction between β_2GPI and ApoER2′ as platelet receptor, the same authors reported that β_2GPI dimers can be co-precipitated with ApoER2′ (Lutters et al., 2003).

5.2. Candidate β_2 glycoprotein I cell receptors on placental tissues

5.2.1. Trophoblast cell receptors

It is widely accepted that β_2GPI can be expressed on trophoblast cell membranes (in particular syncytiotrophoblast). The cationic phospholipid-binding site of the fifth domain of β_2GPI may interact with anionic phosphatidylserine exposed on the syncytiotrophoblast, explaining why this tissue is rich in β_2GPI (reviewed in Di Simone et al., 2007).

Since both annexin A2 and TLR4 have been reported to be expressed on trophoblast cell membrane (Bogic et al., 1999; Abrahams and Mor, 2005), it is useful to speculate on their possible role as candidate receptors for β_2GPI. However, evidence for such a possibility is not available in the literature up to now.

5.2.2. Stromal decidual cells

Stromal decidual cells have recently been recognized to play a role in the pathogenesis of APS pregnancy morbidity (Francis et al., 2006). Moreover, decidua was found to be targeted by aPL in animal models of fetal loss (Girardi et al., 2003). These findings suggest that β_2GPI may be expressed on decidual cells and recognized by β_2GPI-dependent aPL. Our preliminary in vitro studies showed that β_2GPI-dependent aPL react with human stromal decidual monolayers (Borghi et al., 2007). However, the nature of the receptor(s) for β_2GPI and the effects triggered when complexed with the specific antibodies are still a matter of research. By using blocking antibodies against β_2GPI candidate receptors (i.e. TLR4, annexin A2 and ApoER2′) we found a partial inhibition of anti-β_2GPI-mediated binding and cellular activation (Borghi et al., 2007).

These findings suggest that the same cell receptors found on endothelial cells and platelets are involved in decidual cells and that more than one receptor mediates the interaction between β_2GPI and stromal decidual cells (Borghi et al., 2007).

5.3. Other cell types

5.3.1. Renal epithelial cell receptors

Megalin is a renal epithelial endocytic receptor belonging to the LDL receptor family and responsible for the uptake of vitamin D-binding protein and vitamin D in the renal tubules and of sex hormone-binding protein and either androgen or estrogen in the reproductive tissues. It is an efficient and physiologically relevant β_2GPI-binding protein. In fact, megalin-deficient mice show an absent renal uptake of β_2GPI (Moestrup et al., 1998).

5.3.2. Fibroblast receptors

TLR2 has recently been described to be involved in anti-β_2GPI antibody-mediated activation of fibroblasts via a MyD88-dependent pathway (Satta et al., 2007). As TLR2 is also expressed on endothelial cells, monocytes, and platelets and shares in common with TLR4 several ligands including self molecules, it may act as co-receptor in aPL-mediated cell activation.

6. Non-thrombotic antiphospholipid syndrome manifestations

As mentioned earlier, thrombosis cannot explain all the clinical manifestations of the APS and additional pathogenic mechanisms have been suggested to mediate the defective placentation for example (see Sections 3 and 4). Another example of aPL-associated non-thrombotic manifestations is represented by the kidney involvement in APS. In fact, besides thrombosis in medium/large arterial and/or venous renal vessels, aPL have been also associated with kidney lesions characterized by the risk of developing an end-stage insufficiency (APS nephropathy). There are no studies that specifically address the pathogenic mechanisms by which aPL can induce such damage; however, the ability of aPL to perturb endothelium at the level of the kidney microcirculation is likely to play a major role (Amigo, 2006).

Central nervous system (CNS) involvement is another APS condition in which clear ischemic events cannot be identifiable in all the cases (Miyakis et al., 2006). A direct aPL-mediated effect on neuronal cells has been suggested and there is preliminary evidence that the reactivity with β_2GPI expressed on neuronal membranes may play a role (Roldan and Brey, 2007; Lavazza et al., 2007).

Table 2
Mechanisms mediated by antiphospholipid antibodies in atherosclerotic plaque formation

- aPL interact with endothelial cells and monocytes inducing a proinflammatory and procoagulant phenotype
- aPL cross-react with oxidized LDL, favoring their uptake by monocyte/macrophages and foam cell formation
- β_2GPI can be detectable in the atherosclerotic plaques
- Active immunization of ApoER2′-deficient mice with β_2GPI increases the occurrence of atherosclerosis in the animals

Antiphospholipid antibodies may contribute to atherosclerotic plaque formation and this has been shown in various experimental in vitro and in vivo models (Shoenfeld et al., 2005) (Table 2). They may also be responsible for the events upstream of the vessel stenosis and ischemic events themselves.

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CHAPTER 5

Tissue Factor in Antiphospholipid Antibody-induced Pregnancy Loss: Thrombosis versus Inflammation

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1. Antiphospholipid antibodies and thrombosis

The antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL). Antiphospholipid antibody is a term that encompasses a group of heterogeneous, often coexisting antibodies including lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), and antibodies against β_2-glycoprotein I (β_2GPI) alone. Despite the name, these antibodies are not directed against phospholipids, but, rather, target intravascular proteins, either alone or in complex with anionic phospholipids. Most pathogenic aPL, detected either as prolongation of the activated partial thromboplastin time (LAC) or by their ability to bind to cardiolipin-coated wells (aCL), are directed against β_2GPI (Galli et al., 1990; McNeil et al., 1990; Galli et al., 2003). aPL antibodies are widely accepted as pathogenic and are believed to promote thrombosis in several ways (Blank et al., 1991). Knowing that aPL has been shown to be a family of autoantibodies with diverse cross-reactivities, we need to consider the possibility that more than one mechanism/signaling pathway may be involved in thrombosis. Several mechanisms have been proposed (Fig. 1): direct binding of antibodies to endothelial cells, monocytes, and platelets (complement-independent mechanisms) (A1, A2, A3) and indirect pathways (complement-dependent mechanisms) (B1, B2).

2. Direct binding of antibodies: complement-independent mechanisms

A number of in vitro studies have shown that certain aPL antibodies, specifically those directed against β_2GPI, activate endothelial cells (Fig. 1, A1), inducing the expression of procoagulant molecules such as tissue factor (TF) (Amengual et al., 1998; Zhou et al., 2004), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin and endothelin 1 (ET-1) (Simantov et al., 1995; Amengual et al., 1998; Atsumi et al., 1998).

In addition, it has been shown that aPL antibodies against β_2GPI dysregulate the fibrinolytic system by crosslinking or clustering annexin 2 (profibrinolytic endothelial cell surface receptor) on the endothelial surface, inducing a prothrombotic phenotype. Cesarman-Maus and colleagues reported that antibodies directed against annexin 2 are detected in patients with thrombosis and they showed that anti-annexin 2 antibodies induce

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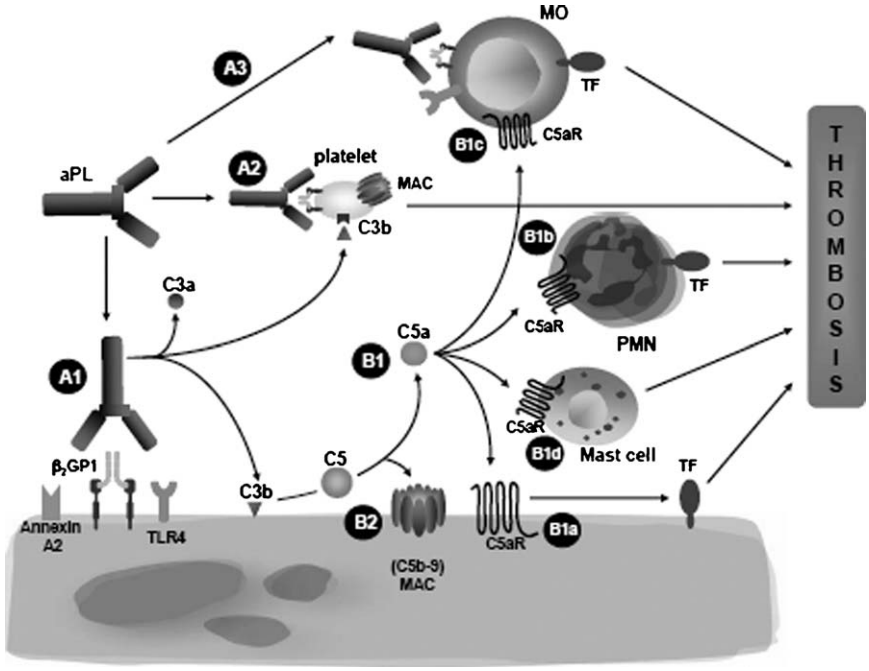

Figure 1. Mechanism/signaling pathways involved in antiphospholipid antibody-induced thrombosis. Several mechanisms have been proposed in aPL-induced thrombosis. Direct binding of antibodies to endothelial cells, monocytes and platelets (complement-independent mechanisms) (A1, A2, A3) and indirect pathways (complement-dependent mechanisms) (B1, B2). C5a can induce thrombosis by interacting with C5aR on different cells: C5a can directly induce expression of tissue factor (TF) on endothelial cells (B1a). Neutrophils (B1b) and monocytes (B1c) can also express TF in response to C5a. C5a can also induce a prothrombotic phenotype in mast cells (B1d). The MAC is a cytolytic pore-forming complex (B2) that triggers endothelial cell and platelet activation.

tissue factor expression on endothelial cells as effectively as that observed by anti-β_2GPI antibodies from patients (Cesarman-Maus et al., 2006). Other authors suggest that because annexin 2 is not a transmembrane protein, this interaction may require a transmembrane "adaptor" protein that is able to mediate intracellular signaling. One candidate is toll-like receptor-4 (TLR4) and this may act as a "co-receptor" for annexin 2 (Zhang et al., 2004). There is some evidence that the MyD88/TRAF6 signaling cascade is triggered by aPL antibodies reacting with β_2GPI on the endothelial cell surface membrane, consistent with the involvement of TLR4. It has been shown that anti-β_2GPI antibodies activate endothelial cells by inducing the nuclear translocation of NF-κB and by the phosphorylation of mitogen-activated protein kinase (MAPK) p38, leading to the expression of proinflammatory cytokines, cellular adhesion molecules, and TF (Pierangeli et al., 2007). De Laat and colleagues demonstrated that anti-β_2GPI antibodies compete with annexin A5 for binding to procoagulant anionic phospholipids, such as phosphotidylserine, thus disrupting an anticoagulant annexin A5 crystal shield on endothelial cells and promoting thrombosis (de Laat et al., 2007). The hypercoagulable state in patients with antiphospholipid syndrome (APS) has also been associated with alterations in the anticoagulant protein C/S pathway (Todorova and Baleva, 2007).

Besides endothelial activation by aPL antibodies, platelets constitute an important target of cellular injury in APS. A study by Jy and colleagues suggested that platelet activation rather than endothelial injury may explain the increased thrombosis in aPL-positive subjects (Jy et al., 2007). As a plasma cationic protein, β_2GPI binds to anionic phospholipids, and more importantly, it can be expressed on the surface of different cell types. Anti-β_2GPI antibodies recognize the

molecule expressed on endothelial cells (Fig. 1, A1), platelets (Fig. 1, A2) and monocytes (Fig. 1, A3). Once bound, the antibodies trigger cell signaling that modulates biological responses potentially responsible for the pathologies associated with APS. Platelets incubated with aPL antibodies exhibited a significant increase in p38 phosphorylation, platelet aggregation, and thromboxane B_2 (TXB_2) production (Pierangeli et al., 2004). In addition, β_2GPI acts as an inhibitor of the intrinsic blood coagulation pathway in vitro (Schousboe, 1985), ADP-mediated platelet aggregation, and the prothrombinase activity of activated platelets (Nimpf et al., 1987). Van Lummel and colleagues demonstrated that dimerized β_2GPI binds and activates apolipoprotein E receptor 2′ (ApoER2′) on platelets and increases platelet adhesion and thrombus formation (van Lummel et al., 2005). Effects of aPL upon platelets have not been completely elucidated. aPL bind anionic phospholipids but they are normally on the inner side of cell membranes. When platelets are activated, anionic phospholipids, such as phosphotidylserine, are exposed. Activated platelets may contribute to thrombosis in APS by persistent exposure of a procoagulant surface.

Monocyte activation is well documented in patients with aPL antibodies. Growing evidence suggests that increased TF activity on circulating blood monocytes is an important mechanism of hypercoagulability in APS and that aPL antibodies are directly responsible. de Prost (1990) reported that monocyte procoagulant activity was increased in patients with aPL antibodies. Several studies have demonstrated that monocytes isolated from APS patients exhibit increased expression of TF mRNA and antigen (Cuadrado et al., 1997; Amengual et al., 1998; Dobado-Berrios et al., 1999). However, the mechanism(s) by which aPL bind to monocytes and trigger intracellular signal transduction, gene transcription, and procoagulant activity are still not fully understood. Studies have demonstrated expression of annexin 2 on the surface of monocytes, suggesting that a mechanism for cellular activation may involve crosslinking or clustering of annexin 2 by either anti-annexin 2 antibodies or anti-β_2GPI (Falcone, 2001). Several TLRs found on endothelial cells are also found on monocytes, suggesting that these receptors could mediate anti-β_2GPI/β_2GPI interactions in both cell types. However, direct binding between β_2GPI and a TLR has not as yet been demonstrated on either cell type. β_2GPI can recognize, bind, and precipitate negatively charged lipid vesicles (Balasubramanian, 1997), and the presence of anti-β_2GPI increases the binding affinity of β_2GPI for phosphotidylserine (Willems et al., 1996). β_2GPI may regulate interactions between phosphotidylserine-expressing cells and macrophages. The causative role of APS patient autoantibodies in monocyte TF expression has been demonstrated in a number of studies, showing that serum, plasma, and purified total IgG from APS patients enhance TF expression and procoagulant activity on monocytes isolated from healthy individuals (Schved et al., 1992; Kornberg et al., 1994; Reverter et al., 1998; Zhou et al., 2004). In particular, anti-β_2GPI human monoclonal antibodies derived from APS patients enhance monocyte TF mRNA and activity in a β_2GPI-dependent fashion (Roubey, 1992; Vega-Ostertag et al., 2005; Cuadrado et al., 2006).

3. Complement-mediated mechanisms

Complement is a core component of the immune system, which performs vital roles in immune surveillance. However, complement components can inappropriately target self tissues and cause pathology. Complement activation has emerged as a common event in the pathogenesis of many diseases, many of them associated with endothelial activation (Makrides, 1998; Tsai, 2006; Markiewski and Lambris, 2007). We have described how complement activation is crucial for aPL-induced pregnancy loss in mice (Holers et al., 2002; Girardi et al., 2003; Girardi et al., 2004; Redecha et al., 2007). Using an in vivo model of thrombosis and microcirculation in which aPL antibodies induce a significant increase in thrombus size and endothelial cell activation we found that complement activation of C3 and C5 are required for aPL-induced enhanced thrombosis (Pierangeli et al., 2005).

There are three pathways of complement activation (Fig. 2): the classical, mannose-binding lectin

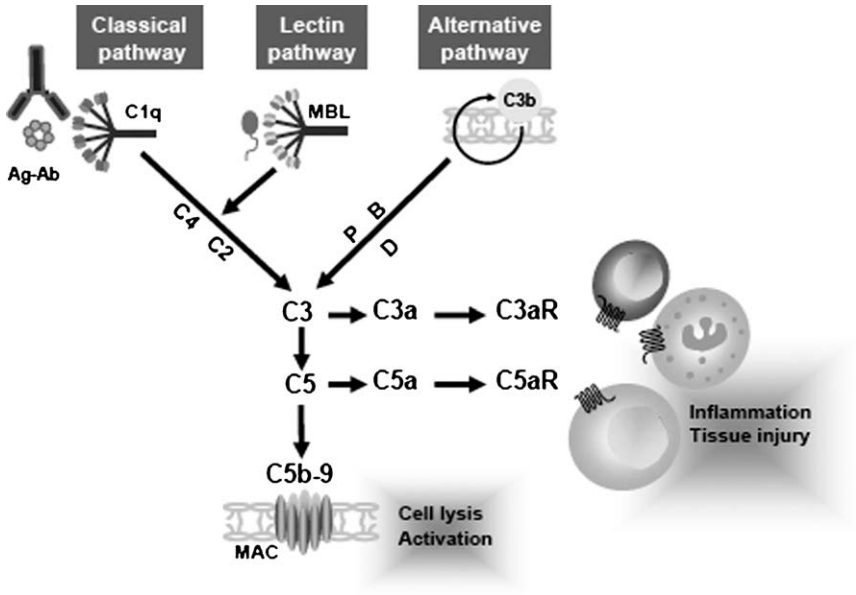

Figure 2. Schematic diagram of the three complement activation pathways and complement fragments that can induce tissue inflammation and tissue injury.

(MBL), and alternative pathways. These three activation pathways lead to cleavage of C3 and generation of the fragments C3a and C3b. C3a, an anaphylatoxin that binds to receptors on leukocytes and other cells, causes activation and release of inflammatory mediators. C3b is covalently bound to the site of complement activation and then forms C5 convertase enzymes. C5 convertases cleave C5, generating C5b and anaphylatoxin C5a. C5a is a potent soluble inflammatory anaphylatoxic and chemotactic molecule that recruits and activates neutrophils and monocytes and mediates endothelial cell activation through its receptor, C5a receptor (C5aR). Binding of C5b to the target initiates the non-enzymatic assembly of the membrane attack complex (MAC). MAC is a pore-forming lipophilic complex that can destroy cells by permeabilization of the membranes and can also trigger cell activation.

3.1. How complement contributes to thrombosis

After aPL binding, the complement system is activated. C3 is cleaved, generating C3a and C3b. C3b binds to P-selectin. P-selectin contains structural motif present in many complement-binding proteins, such as complement receptors (CR1 and CR2). It is expressed on platelets by C1q, an initiator of the classical pathway of complement activation. By this mechanism platelet activation leads to activation and propagation of the complement system. These findings point out an important mechanism by which inflammation may localize to sites of thrombosis (Del Conde et al., 2005). Once the C5 convertase is formed, C5 is cleaved generating C5a (Fig. 1, B1). C5a can induce thrombosis by interacting with C5aR on different cells: C5a can directly induce expression of TF on endothelial cells (Fig. 1, B1a) (Muhlfelder et al., 1979). The induction of TF on endothelial cells by C5a may represent an important interrelationship between the inflammatory and coagulation schemes. Neutrophils (Fig. 1, B1b) and monocytes (Fig. 1, B1c) can also express TF in response to C5a (Semeraro et al., 1985; Ritis et al., 2006; Redecha et al., 2007). C5a induces release of secretory granules, particularly in neutrophils, leading to extracellular liberation of inflammatory mediators including elastase, peroxidase, and glucuronidase, inducing tissue injury. These

enzymes not only induce inflammation but directly alter platelet function and/or participate in coagulation cascade reactions on platelet or neutrophil surfaces to enhance fibrin formation (Goel and Diamond, 2003). Complement component C5a can also induce switch in mast cells (Fig. 1, B1d) from a profibrinolytic to a prothrombotic phenotype, by upregulating plasminogen activator inhibitor (PAI-1) (Wojta et al., 2002, 2003). Increased mast cells and increased levels of C3 are observed in coronary artery thrombosis (Shebuski and Kilgore, 2002). Tumor necrosis factor alpha (TNF-α), released from different cells upon stimulation by C5a (Berman et al., 2005), can activate endothelial cells and induce TF expression and thrombosis (Bevilacqua et al., 1986; Parry and Mackman, 1995; Karmann et al., 1996; Miller et al., 1998). As can be observed in Fig. 1, several types of inflammatory cells can be recruited and activated either directly or indirectly by C5a to cause thrombosis and tissue injury. Formation of the MAC (Fig. 1, B2) involves the sequential assembly of the five terminal complement proteins (C5–C9) into a heteropolymeric complex. The MAC is a cytolytic pore-forming complex that can destroy cells by permeabilizing the plasma membrane. Although MAC may also cause tissue necrosis by lyzing cells, non-lethal effects of the MAC which trigger cell activation are likely to be more important to human pathology (Morgan, 1989; Shin et al., 1996). Incorporation of the MAC into the cell membrane activated platelets and results in the exposure of procoagulant lipids (Sims and Wiedmer, 1991), the release of microparticles (MP), providing an extra surface for the conversion of prothrombin to thrombin through prothrombinase (Sims et al., 1988), and granule secretion from the cytoplasm of the platelets (Ando et al., 1988). It has been reported that MAC activates endothelial cells through the autocrine effects of IL-1α, causing expression of TF, PAI-1, chemokines and adhesion molecules (Saadi et al., 2000). The complement regulatory protein CD59 interferes with assembly of the MAC preventing the generation of the pore. Because the MAC may contribute to thrombosis, CD59 may be important in protecting glomerular endothelium from thrombosis. Mice have two CD59 genes (mCD59a and mCD59b); mCD59b-knockout mice have a phenotype characterized by hemolytic anemia and platelet activation (Qin et al., 2003). Blockade of CD59 with a monoclonal antibody was associated with more C5b-9 formation in glomeruli and increased platelet and fibrin deposition in a rat model of immune thrombotic microangiopathy (Nangaku et al., 1998).

3.2. Coagulation proteases and cellular signaling

The coagulation cascade is assembled on membrane surfaces due to the binding of factor VII/VIIa (FVII/VIIa) to TF, which is a transmembrane glycoprotein, and the interaction of positively charged γ-carboxyglutamic acid (Gla) domains of coagulation proteins with negatively charged phospholipids, such as phosphotidylserine, that are exposed after cellular activation and/or apoptosis (Mann et al., 1988). The two major protease complexes of the coagulation cascade are the initiating complex (TF:FVII/FVIIa) and the prothrombinase complex (FVa:FXa) (Edgington et al., 1991). In addition, free FXa and thrombin are generated during the activation of the clotting cascade. Coughlin and colleagues were the first to isolate and clone the receptor that mediates cellular activation in response to thrombin (Vu et al., 1991). Initially, this receptor was simply called the thrombin receptor but this name has been changed to protease-activated receptor 1 (PAR-1). As its name suggests, the extracellular domain of PAR-1 is cleaved by thrombin to generate a new N-terminus that acts as a tethered ligand and activates the receptor. Other members of the PAR family have been identified and it now possesses four members (Fig. 3) (Coughlin, 2000; Major et al., 2003).

PAR-1 is activated by low concentrations of thrombin whereas higher concentrations are required to activate PAR-3 and PAR-4. FXa also activates PAR-1. PAR-2 is activated by a variety of proteases that include FVIIa and FXa (Ruf et al., 2003). Therefore, the clotting cascade not only functions to generate fibrin but also activates a variety of cells by proteolytic cleavage of PARs.

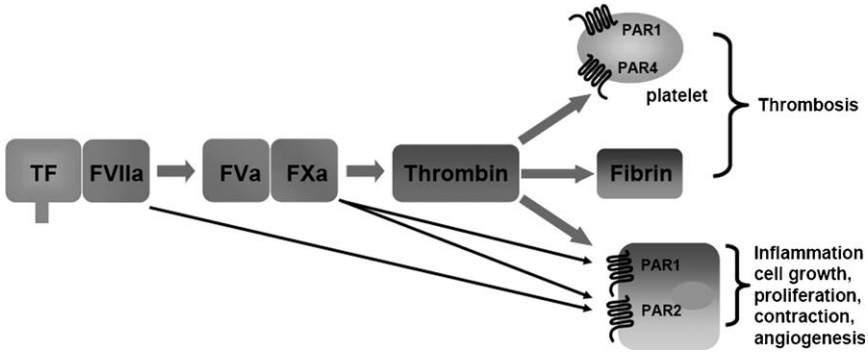

Figure 3. Members of the protease-activated receptor (PAR) family. Four PARs have been identified by molecular cloning. A wide range of proteases cleave and activate PARs, including proteases from the coagulation cascade, inflammatory cells, and the digestive tract. Therefore, the clotting cascade not only functions to generate fibrin but also activates a variety of cells by proteolytic cleavage of PARs. Receptor activation initiates an array of signaling events in many cell types with diverse consequences like hemostasis, inflammation, cell growth, cell contraction, and angiogenesis. PAR-1, formerly known as the thrombin receptor, is activated by low concentrations of thrombin and factor Xa (FXa). PAR-2 is activated by a variety of proteases that include FVIIa and FXa. PAR-1 and PAR-2 are localized in many different cells, endothelial cells, myeloid cells, fibroblasts, myocytes, and neurons among others. PAR-3 and PAR-4 are activated by thrombin. Thrombin activates human platelets by cleaving both PAR-1 and PAR-4. However, mouse platelets do not express PAR-1 and are activated by docking of thrombin onto PAR-3 and cleavage of PAR-4. TF, tissue factor.

The best-studied cellular activation by coagulation proteases is that of platelets. Thrombin activates human platelets by cleaving both PAR-1 and PAR-4. However, mouse platelets do not express PAR-1 and are activated by docking of thrombin onto PAR-3 and cleavage of PAR-4 (Coughlin, 2000). Each of the different PARs has been knocked out in mice (Connolly et al., 1996; Darrow et al., 1996; Kahn et al., 1998; Nierodzik et al., 1998; Damiano et al., 1999; Lindner et al., 2000; Sevastos et al., 2007). PAR-1 deficiency is associated with a 50% intrauterine lethality but surviving mice lacking PAR-1 are normal. Mice deficient in PAR-2, PAR-3, or PAR-4 are normal. Analysis of the various knockout mice in different models has shown that PARs are involved in many different responses. However, it is often difficult to determine which of the many proteases that can activate the PARs are responsible for their activation in vivo.

In addition to initiating the extrinsic pathway of coagulation, TF contributes to inflammation. TF expression is a characteristic feature of acute and chronic inflammation in conditions such as Crohn's disease, sepsis, glomerulonephritis, atherosclerosis, ischemia/reperfusion, and rheumatoid arthritis (Tremoli et al., 1999; Bokarewa et al., 2002; Matsuyama et al., 2003; Pawlinski et al., 2004; Cunningham et al., 2004; Anthoni et al., 2007). This review describes the role of TF in the inflammatory reaction that leads to fetal injury and pregnancy loss induced by aPL antibodies.

3.3. Tissue factor enhances inflammation in aPL-induced pregnancy

The APS is characterized by increased risk of vascular thrombosis involving venous, arterial, and placental circulations. When thrombosis occurs in the placenta, it is associated with poor obstetrical outcomes, including fetal death and growth restriction. Although it is clear that the specific antigenic reactivity of aPL antibodies is critical to their effect, the pathogenic mechanisms that result in thrombosis and tissue injury in vivo are incompletely understood. To better understand these mechanisms, we developed a mouse model of aPL-induced pregnancy loss. In this model, passive transfer of human and murine aPL antibodies induce fetal loss and growth restriction, thereby demonstrating the direct pathogenic role for aPL antibodies (Holers et al., 2002; Girardi et al.,

2003). Using this model of APS we have shown that complement activation plays an essential and causative role in fetal loss and tissue injury. Specifically, we have identified the proinflammatory sequelae of C5a–C5aR interactions and the recruitment of neutrophils as the critical intermediates linking pathogenic aPL antibodies to fetal damage (Girardi et al., 2003). APS is considered a thrombophilic disorder. However, animal studies from our laboratory have shown the importance of inflammation in APS (Holers et al., 2002; Girardi et al., 2003, 2004). Recently, human studies have shown that inflammation in the placenta may contribute to APS pregnancy complications, reinforcing this new concept of the APS as an inflammatory disorder (Stone et al., 2006).

Thrombosis and inflammation are linked in many clinical conditions (Esmon, 2005). TF, the major cellular initiator of the coagulation protease cascade, plays important roles in both thrombosis and inflammation (Chu, 2006). Besides initiating the extrinsic pathway of the coagulation cascade, TF also contributes to inflammation. TF complexes (TF:FVIIa and TF:FVIIa:FXa) induce the expression of TNF-α, interleukins, and adhesion molecules by cleaving PARs (Camerer et al., 2000; Ruf et al., 2003; Strukova, 2006). Knowing that TF expression is increased in APS, we studied whether TF contributes to aPL-induced fetal loss in mice. Blockade of TF activity with a specific monoclonal antibody, 1H1 (Kirchhofer et al., 2005), and experiments in low TF-expressing mice (Pawlinski et al., 2004) showed less inflammation (fewer neutrophils and less C3 deposition) in decidua and increased embryo survival in aPL-treated mice, suggesting that TF is a proinflammatory molecule in this model (Redecha et al., 2007).

Experiments performed in $TF^{floxed/floxed}$/LysM-Cre mice that do not express TF on myeloid cells allowed us to distinguish the role of trophoblast TF from that of myeloid cell TF. The protection from aPL-induced pregnancy loss observed in these mice emphasizes the crucial role of TF in maternal myeloid cells (Redecha et al., 2007). Moreover, knowing that monocytes are not required for aPL-induced pregnancy loss and that neutrophils from aPL-treated $TF^{floxed/floxed}$/LysM-Cre mice do not express TF allows us to conclude that TF expression on maternal neutrophils plays a causative and crucial role in aPL-induced fetal injury. $TF^{floxed/floxed}$/LysM-Cre mice treated with aPL showed normal pregnancies and diminished decidual inflammation, suggesting that TF expression on neutrophils modulates the ability of the neutrophil to induce tissue injury. Indeed, neutrophils from $TF^{floxed/floxed}$/LysM-Cre mice treated with aPL showed a lower generation of oxidants and less free radical-mediated lipid peroxidation in decidua when compared with $TF^{floxed/floxed}$ control mice that express TF, suggesting that TF is a modulator of oxidative burst in neutrophils (Redecha et al., 2007).

Hirudin and fondaparinux did not prevent pregnancy loss in this mouse model of APS (Girardi et al., 2004), suggesting that anticoagulation is not sufficient to prevent miscarriages. These anticoagulants inhibit coagulation at the level of thrombin and FXa respectively, but they do not inhibit the formation of the TF:FVIIa and TF:FVIIa:FXa complexes. These complexes can activate G protein-coupled PARs and induce inflammation (Ruf et al., 2003). Agonists of PARs, notably of PAR-2, induce inflammation in many diseases associated with neutrophil infiltration and alterations in epithelial permeability (Lindner et al., 2000). Therefore, TF-mediated signaling through PARs may promote inflammation, leading to trophoblast injury and pregnancy loss.

Collectively, our study demonstrates that maternal TF on neutrophils is essential in the pathogenesis of aPL-induced fetal loss and reveals a functional linkage between complement components, TF, and neutrophils. We propose that after aPL IgG binding to trophoblasts, complement activation occurs, leading to generation of the anaphylatoxin C5a, which attracts and activates neutrophils through C5aR (Fig. 4). C5a–C5aR interaction on neutrophils results in TF expression. Activated neutrophils release reactive oxygen species and proteolytic enzymes leading to decidual damage. TF expression on neutrophils appears to lead to the formation of complexes that amplify inflammation, cell injury, and fetal death.

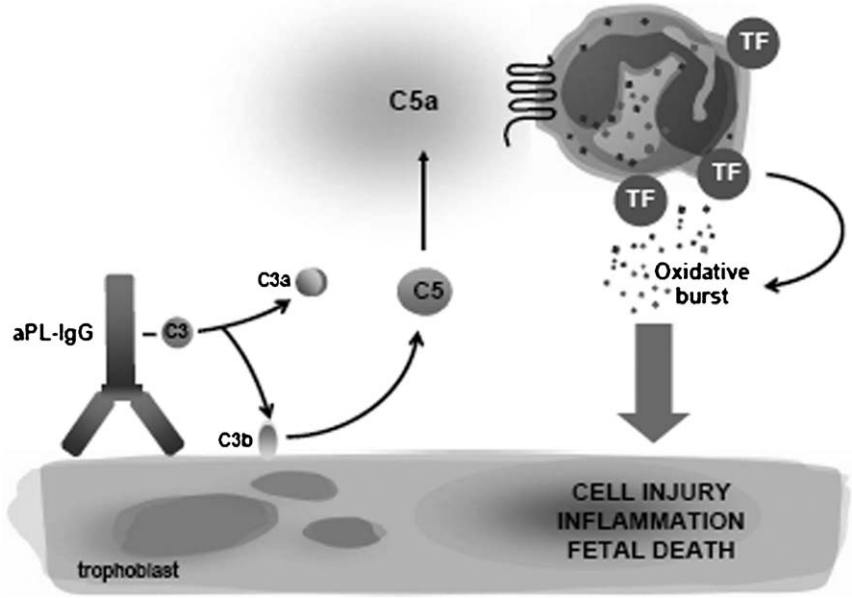

Figure 4. Proposed mechanism of antiphospholipid antibody-induced tissue factor (TF) increase and fetal death. aPL bind to trophoblasts where they activate the complement cascade, leading to the generation of C5a. The engagement of C5a with its receptor C5aR on neutrophils results in TF expression. TF on neutrophils increases cellular activation (reactive oxygen species (ROS) production), leading to inflammation, decidual injury, and fetal death.

The identification of TF as an important mediator of C5a-induced oxidative burst in neutrophils in aPL-induced fetal injury provides a new target for therapy to prevent pregnancy loss in the APS.

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CHAPTER 6

Genetic Aspects of the Antiphospholipid Syndrome: HLA Associations

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1. Human leukocyte antigen (HLA) associations

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (aPL) in serum together with clinical manifestations such as thrombosis (both arterial and venous), fetal losses, hemolytic anemia, and thrombocytopenia. There is little doubt as to the pathogenic role of aPL in determining the clinical manifestations of APS, even if their mechanism of action has not been fully clarified.

The etiology of APS, however, is still unknown. Like many other autoimmune diseases, this syndrome arises in a predisposed subject after antigenic stimuli from various sources. Proof of the genetic predisposition of APS lies in the observation of familial clustering of cases, greater prevalence of aPL in the serum of subjects sharing the same descent of patients, animal models (mice), and associations with human leukocyte antigen (HLA) alleles.

Many autoimmune diseases are associated with genes in the MHC (major histocompatibility complex) region. In some autoimmune disorders, such as systemic lupus erythematosus (SLE), MHC antigens seem to be associated with specific autoantibodies, including anticardiolipin (aCL) and anti-β_2 glycoprotein I (anti-β_2GPI), rather than with the disease itself (Smolen et al., 1987; Lulli et al., 1991). Thus, it appears that MHC genes may influence not only the expression of autoimmune diseases, but also the production of autoantibodies that can be found in these diseases.

Many researchers in the field of immunogenetics have investigated possible associations between APS or the various antibodies directed against negatively charged phospholipids and MHC genes or their products. However, there is increasing evidence that aPL represent a heterogeneous group of antibodies, which includes lupus anticoagulant (LAC), aCL, anti-β_2GPI, antibodies to prothrombin, annexin V, phosphatidylethanolamine, phosphatidylserine and other oxidized phospholipids. Thus, it appears evident that the spectrum of HLA associations with APS might become clearer if more specific autoantibody subgroups are studied.

In this chapter, studies on the immunogenetic predisposition to APS and to the production of the aPL are summarized and discussed.

2. HLA, antiphospholipid syndrome, and antiphospholipid antibodies

The question of whether a genetic predisposition to develop APS and to produce aPL exists can be examined both in animal models and in humans.

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The presence of aPL has been reported in some, but not all, SLE-prone mice. They can be detected in MRL/MP/lpr/lpr (MRL/lpr) and $MRL^{+/+}$ mice (Gharavi et al., 1989) as well as in NZW × BXSB F1 mice (Hashimoto et al., 1992), but NZB × NZW F1 mice, a classical murine model of SLE, do not develop these autoantibodies. These aPL can also be spontaneously produced in normal C57BL/6 J mice, and estrogen treatment of these mice augments the incidence as well as the levels of these autoantibodies (Ansar Ahmed and Verthelyi, 1993). It thus appears that the genetic background of mice can influence the production of aPL, and this production can be modulated by hormones. Nevertheless, it has not been clarified whether aPL are constitutively expressed by mice or induced by antigenic stimulation. There is a preferential usage of certain Vh and Vk chains in aCL of NZW × BXSB F1 mice (Hashimoto et al., 1992). This fact could indicate that in these mice aCL are not germline encoded but antigen driven.

Using microsatellite markers in the NZW × (NZW × BXSB) F1 backcross male progeny, Ida et al., mapped BXSB alleles contributing to the generation of aCL, platelet-binding antibodies, thrombocytopenia, and myocardial infarction (Ida et al., 1998). They found that the generation of each disease character was controlled by two major independently segregating dominant alleles, and that a combination of the two alleles appeared to produce full expression of each character, as a complementary gene action. This finding suggests that no single factor, such as aCL, can explain the pathogenesis of APS. Rather, a combination of susceptibility alleles characterizes unique features in male (NZW × BXSB) F1 mice that are prone to develop APS.

In humans, the contribution of immunogenetics to the development of aPL and APS has been addressed mainly by family studies and by population studies looking at the HLA region. The APS may exist both as a primary condition as well as in the setting of another autoimmune disease (mainly SLE), and this implies possible differences in the association with HLA. Furthermore, aPL are a heterogeneous family of autoantibodies. Some aPL can be found in autoimmune diseases, but others appear during the course of infectious diseases, neoplasias, or are drug-related; they can also be present as an isolated phenomenon in healthy individuals. Their presence is not always associated with the clinical manifestations of APS, and even in experimental animal models not all aPL are of pathogenetic significance. Some aPL bind preferentially to anionic phospholipids, whereas others react with zwitterionic phospholipids, and their binding can be either enhanced or depressed by β_2GPI, depending on the source of aPL. Therefore, what we call ''antiphospholipid antibodies'' may comprise a group of antibodies whose unique common feature is their reactivity against phopsholipids, but with different specificities and different HLA associations.

HLA alleles can be detected by conventional serologic methods or by molecular methods, such as restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR)-amplified HLA genes and DNA typing by oligonucleotide hybridization. Molecular methods allow better definition of the various polymorphisms.

2.1. Family studies

Familial occurrence of aPL with or without clinical evidence of APS has been documented since 1980.

Exner et al., described three sets of siblings with LAC, two of which had more than one clinically affected member (Exner et al., 1980). Matthey et al., described a family with primary APS, consisting of four affected members (Matthey et al., 1989), and Jolidon et al., reported a family with three cases of primary APS (Jolidon et al., 1991).

Various studies examined HLA by serological methods. Dagenais et al., described an English Canadian family in which aCL were associated with a spectrum of clinical manifestations, from asymptomatic carriers to the typical thrombotic disease in association with SLE and autoimmune thyroid disease (Dagenais et al., 1992). They found the paternal haplotype A30; Cw3; B60; DR4; DRw53; DQw3 to be associated with aCL. The occurrence of LAC in families carrying haplotypes that contained either DR4 or DR7 also has been reported by others (Rouget et al., 1982; Mackie et al., 1987).

May et al., have described a family, including identical twins and their mother, in which all members had SLE and presented with different manifestations of APS (May et al., 1993). The mother and the twins shared the HLA haplotype that included DR4, DRw53, and DQw7, whereas C4A or C4B deficiencies could not be implicated in the autoimmune process.

In conclusion, family studies suggest a genetic predisposition to APS, either when it presents as a primary condition or when it is seen in the context of SLE. It appears that this genetic predisposition is in part accounted for by the HLA system, the most consistent associations being those with DR4 and DRw53. Furthermore, it appears that LAC and aCL are both associated with the same HLA antigens, even if these two methods of detecting aPL do not overlap completely.

2.2. Population studies on primary antiphospholipid syndrome

In a study of primary APS and HLA associations, as detected by molecular methods, HLA-DQw7 (DQB1∗0301 allele) was significantly associated with disease. All patients with DQw7 were HLA-DR4 or -DR5-positive (Arnett et al., 1991).

Asherson et al., reported on 13 English patients with primary APS, in which both class II genes and class III genes were examined by molecular methods (Asherson et al., 1992a). They found that significant differences were limited to the HLA class II region of the MHC. In fact, DR4 and DRw53 were found with increased frequency in patients compared with controls, whereas DR3 was absent in all patients. No significant associations between any DQB alleles or C4 or 21-hydroxylase gene polymorphisms and primary APS were found, although DQw7 was not significantly increased in patients. More recently, Caliz et al., found that the haplotypes DQB1∗0301/4-DQA1∗0301/2-DRB1∗04 and DQB1∗0604/5/6/7/9-DQA1∗0102-DRB1∗1302 were more frequent in 53 white British patients with the primary APS than in controls (Caliz et al., 2001). The most striking association was found between DQB1∗0604/5/6/7/9-DQA1∗0102-DRB1∗1302 and anti-β_2GPI in primary APS. The DQB1∗0301/4-DQA1∗0301/2-DRB1∗04 haplotype was also associated with antiphosphatidylserine/prothrombin autoantibodies in the same group of patients (Bertolaccini et al., 2000). In another study on the same patients, Bertolaccini et al., evaluated the role of tumor necrosis factor-alpha (TNF-α), an immunomodulatory cytokine with prothrombotic action, encoded at the *TNFA* locus in the MHC class III region (Bertolaccini et al., 2001). They found significantly higher plasma TNF-α levels in patients with APS when compared with controls. In addition, they found a strong association between TNFA-238∗A polymorphism and APS, and a possible association of the TNFA-238∗A-DQB1∗0303-DRB1∗0701 haplotype with APS. However, they failed to demonstrate correlation between TNFA-238∗A and plasma TNF-α levels, suggesting that this polymorphism is not implicated in the elevation of TNF-α levels found in APS. It is possible that TNFA-238∗A polymorphism is associated with APS because of its linkage with DRB1∗0701-DQB1∗0303 haplotype. Another study reports the association of HLA-DR5 with primary APS in Mexican patients (Vargas-Alarcon et al., 1995).

To assess whether the MHC profile of patients presenting with primary APS is different from that of patients with secondary APS, Freitas et al., studied 123 patients, 34 of whom presented primary APS and 35 secondary APS due to SLE, 54 SLE patients without APS, and 166 controls. Compared with controls, primary APS patients exhibited a non-significantly increased frequency of DRw53-associated alleles, and secondary APS patients presented an increased frequency of HLA-DRB1∗03 alleles. In addition, HLA-DRB1∗03 alleles were over-represented in secondary APS patients presenting aCL, in SLE patients as a whole, and in SLE patients without APS. Taken together, their results suggest that the HLA class II profile of primary APS is different from that of secondary APS (Freitas et al., 2004).

Sanchez et al., examined the susceptibility of the polymorphisms at the HLA-DM locus (whose products are involved in the antigen processing pathway of HLA class II restricted antigen presentation) to aPL production in a white British population, and observed the skewed distribution

of DMA alleles, including the increase of DMA*0102 in patients with aPL. However, this association could simply reflect the strong linkage disequilibrium between HLA-DM alleles and HLA-class II alleles (Sanchez et al., 2004).

Thus, population studies suggest that HLA genes have a role in conferring susceptibility to develop primary APS. DRB1*04, DR7, DRw53, DQB1*0301/4, DQB1*0604/5/6/7/9, DQA1*0102, and DQA1*0301/2, appear to be the relevant loci. HLA-DR4 seems to be more important in Anglo-Saxon populations, whereas DR7 emerges in populations of Latin origin. Results of those studies in which HLA polymorphisms have been investigated by molecular methods overlap those obtained by serological typing. It is difficult to discriminate whether DR loci contribute to this genetic susceptibility more than DQ loci, because they are in strong linkage disequilibrium.

2.3. Population studies on antiphospholipid antibodies in diseases other than primary antiphospholipid syndrome

Most of these studies deal with SLE and aCL, probably because aCL are more easily detectable than LAC. They are summarized in Table 1.

We recently performed a very large study on about 600 patients with SLE, all of European origin (Galeazzi et al., 2000), analyzing the association of aCL and anti-β_2GPI with HLA class II alleles. Our data showed that aCL are positively associated with HLA-DRB1*04, -DRB1*07, -DQA1*0201, -DQA1*0301, -DQB1*0302, -DRB3*0301, and that anti-β_2GPI have a positive association with DQB1*0302. DQA1*0501 and DRB3*0202 showed a negative association with aCL. For the first time it was demonstrated that aCL and anti-β_2GPI are associated with HLA-DRB1*0402 and -DRB1*0403, among the alleles of the DRB1*04 series. Indeed, DRB1*0402 carried the highest relative risk for the presence of both aCL ($RR = 8.1$) and anti-β_2GPI ($RR = 4.6$), and it was noteworthy that 75% of patients carrying the DRB1*0402 allele were aCL-positive.

We could not find any association with alleles at DRB4 locus (DRw53), and we found that aCL are associated with DR4 in SLE patients both from Spain and Italy, two Latin countries. Thus, it can be argued that both DR4 and DR7 are independently associated with aCL, and that aCL in patients with SLE are associated with alleles at DRB1 locus but not with those at DRw53 locus. According to our results it seems that DRB1*0402 and DRB1*0403 are slightly more important than DR7 and that the association with DRw53 is only apparent because patients typing positive for DRw53 possess haplotypes that also contain either DR4 or DR7.

In addition, we found that aCL and some clinical manifestations shared the same HLA association. This was the case of the association of IgA aCL and Raynaud's phenomenon with DRB1*07 and DQA1*0301, of hemolytic anemia and IgM aCL with DQA1*0301 and of thrombocytopenia and IgG aCL with DRB3*0301. Therefore we can speculate that the association of HLA alleles with particular clinical manifestations of APS we found in this study might be a consequence of the association of these alleles with aCL and/or anti-β_2GPI.

In another paper, we have analyzed whether HLA-DPB1 alleles contribute to the genetic predisposition to develop APS and aPL (aCL and anti-β_2GPI) in the same European cohort of patients with SLE. Our data showed that aCL are positively associated with HLA-DPB1*1501 and -DPB1*2301, and that anti-β_2GPI have a positive association with HLA-DPB1*0301 and -DPB1*1901 (Sebastiani et al., 2003).

More recently, we have evaluated the clinical and HLA class II allele associations of other aPL, such as antibodies antiprothrombin (anti-PT), anti-annexin V (anti-AnnV), anti-protein C (anti-PC) and anti-protein S (anti-PS), in a homogeneous group of 136 European patients with SLE (Sebastiani et al., 2008).

We found an interesting association between anti-PT and the HLA-DQB1*0301;DQA1*03;DRB1*04 haplotype. We have already shown that anti-β_2GPI antibodies are associated with these same alleles in patients with SLE. It is widely accepted that antibodies to PT and anti-β_2GPI are two major autoantibodies responsible for LAC activity, anti-PT responsible for PT-dependent LAC, and anti-β_2GPI for β_2GPI-dependent LAC.

Table 1
Association between HLA alleles and anticardiolipin antibodies in various diseases

Disease	HLA	Frequency[a]	Ethnic origin	Reference
SLE	C4Q0	92	African-American	Wilson et al. (1988)
CBFP	C4Q0	71	Swedish	Stephansson et al. (1993)
SLE	C4A	No association	American	Petri et al. (1993)
SLE	DR7	61	Northern Italian	Savi et al. (1988)
SLE	DR4	87	English	McHugh and Maddison (1989)
Possible primary APS[b]	DR4	56	Australian	McNeil et al. (1990)
	DRw53	83		
SLE	DR4, DR7, DRw53	81	White	Hartung et al. (1992)
SLE	DRB1∗0901	41	Japanese	Hashimoto et al. (1998)
SLE	DR, DQ	No association	White and African American	Gulko et al. (1993)
SLE	DR	No association	Central Italian	Sebastiani et al. (1991)
SLE	DPB1∗1401,0301	45	Central Italian	Galeazzi et al. (1992)
SLE	DRB1∗0402/3,DRB1∗07, DQA1∗0201,DQA1∗0301, DQB1∗0302,	75/56,36 36,47 45	White	Galeazzi et al. (2000)
SLE	DPB1∗1501, ∗2301	50,63	White	Sebastiani et al. (2003)
PSS	DR	No association	American	Asherson et al. (1992b)
JCA	A,B,C,DR	No association	Canadian	Malleson et al. (1992)

Note: SLE, systemic lupus erythematosus; CBFP, chronic biologically false-positive reactors (some affected by SLE); APS, antiphospholipid syndrome; PSS, primary Sjögren's syndrome; JCA, juvenile chronic arthritis.

[a] Frequency of HLA allele in aCL-positive patients (%).

[b] Patients with aCL and occlusion of coronary artery bypass grafts.

For this reason, our observation that anti-PT and anti-β_2GPI share a common genetic background is of interest. In addition, we found that anti-AnnV are positively associated with HLA-DRB1∗08 and negatively associated with -DQA1∗0102; anti-PS were positively associated with -DQB1∗0301. These associations need confirmation in other studies, because they have never been reported and appear to be weak. However, they reinforce the hypothesis that aPL production is under genetic control.

Arnett and colleagues analyzed the association of anti-β_2GPI with HLA class II alleles in three ethnic groups: Mexican Americans (41 patients), white Americans (122 patients), and black Americans (99 patients) (Arnett et al., 1999). The authors examined a rather etherogeneous group of patients affected by primary APS, SLE, and other connective tissue diseases. They found that HLA-DR4 haplotypes, especially those carrying HLA-DQ8 (DQB1∗0302), are strongly associated with anti-β_2GPI in white populations and Mexican Americans, and less so in black populations, who normally have low frequencies of these alleles. In addition, they found that the HLA-DRB1∗1302; DQB1∗0604/0605 haplotype was associated with anti-β_2GPI primarily in black populations, a result similar to that recently reported by Caliz et al., in a completely different ethnic group, white British patients with primary APS (Caliz et al., 2001). In addition, Arnett et al., found a strong negative association between HLA-DR2 (DRB1∗1501/∗1503); DQ6 (DQB1∗0602) and anti-β_2GPI, thus confirming the results of previous studies that had shown a high prevalence of HLA-DR2 (DRB1∗15) in lupus patients (Tiwari and Terasaki, 1985) but not in SLE patients with anti-β_2GPI. We observed similar results in the European sample of lupus patients, where DRB1∗15 was found to be increased in patients with SLE, but not in anti-β_2GPI-positive ones, suggesting a neutral effect, more than a protective role, of this allele on the production of these autoantibodies. Indeed it is more likely that in Europeans the association of DR2 was with SLE itself or with other autoantibody specificities.

Summarizing, the majority of the reports on SLE seem to indicate that aPL are associated with DR4, DR7, the closely linked antigen DRw53, and

Table 2
Association of HLA alleles with anti-β_2 glycoprotein I and antiphosphatidylserine/prothrombin antibodies in SLE and primary APS

Disease	aPL	HLA	Frequency[a]	Ethnic origin	Reference
Primary APS	anti-β_2GPI	DRB1∗1302-DQA1∗0102-DQB1∗0604/5/6/7/9	14	British white	Caliz et al. (2001)
SLE	anti-β_2GPI	DRB1∗0402/3	67/56	European	Galeazzi et al. (2000)
		DQB1∗0302	50		
Primary APS + SLE[b]	anti-β_2GPI	DQB1∗0302	32	White American	Arnett et al. (1999)
Primary APS + SLE[b]	anti-β_2GPI	DR4-DQB1∗0302	64-64	Mexican American	Arnett et al. (1999)
Primary APS + SLE[b]	anti-β_2GPI	DRB1∗1302-DQB1∗0604/5	36-36	Black American	Arnett et al. (1999)
Primary APS	aPTS/PT	DRB1∗04-DQA1∗0301/2-DQB1∗0301/4	31-31-35	British white	Bertolaccini et al. (2000)
SLE	anti-β_2GPI	DPB1∗0301,∗1901	28,67	White	Sebastiani et al. (2003)
SLE	aPT	DQB1∗0301-DQA1∗03-DRB1∗04	19-31-29	White	Sebastiani et al. (2008)

Note: SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; aPL, antiphospholipid antibodies; aPT, antiprothrombin antibodies; aPTS/PT, antiphosphatidylserine/prothrombin antibodies.

[a] Frequency of HLA allele/haplotype in aPL-positive patients (%).

[b] Forty-eight patients affected by primary APS, 196 patients affected by SLE, 18 patients affected by other connective tissue diseases (of whom 4 with APS).

DQB1∗0302 (Tables 1 and 2). The association of aCL with C4A- or C4B-null alleles is less evident, and it may be of some importance only in black American populations. In addition, it appears that the disease itself may influence this aCL–HLA association, since no association can be found in diseases other than SLE.

3. Conclusions

Genetic factors are important in the development of APS. This is demonstrated by animal models, by the familial occurrence of this syndrome, and by its association with various HLA alleles. We favor the hypothesis that the association of APS with HLA alleles is a consequence of the association of aPL with HLA alleles. Some HLA alleles carry the risk to produce aPL, and this is independent of the clinical context. In fact, we find the same associations between HLA and aPL in primary APS and in APS secondary to SLE. The association of HLA-DR4, -DR7, -DRw53, and -DQB1∗0302 with aCL that has been demonstrated in primary APS, can also be found in SLE, a disease with a completely different pattern of HLA allele association (DR2, DR3, DRw52). In addition, the various aPL aCL, LAC, anti-β_2GPI, antiphosphatidylserine/prothrombin antibodies show similar HLA association, again independent of the clinical context (primary APS or SLE), and across various ethnic groups.

It is therefore reasonable to think that, like in SLE, HLA alleles account only in part for the genetic susceptibility to develop APS. In fact, it appears that HLA alleles only determine the susceptibility to produce aPL, which are responsible for the clinical manifestations of APS. Other genes, outside the MHC, give their contribution to the development of this autoimmune syndrome.

For example, it has been shown that a polymorphism in domain 5 of anti-β_2GPI, valine instead of leucine at position 247, is correlated with anti-β_2GPI production in patients with primary APS (Hirose et al., 1999; Atsumi et al., 1999). Amino acid differences of β_2GPI can affect the nature of conformational alterations induced by interaction of this protein with phospholipids. The position 247 polymorphism can affect the conformational change of β_2GPI and the exposure of the epitopes for anti-β_2GPI. Furthermore, additional genetic risk factors for thrombosis have been described in patients with APS, such as factor V Leiden, methylenetetrahydrofolate reductase, and protein C or protein S deficiency. The role of these genetically determined factors in APS is not completely clarified, but it appears that they can act as additional (to aPL) thrombogenic risk factors.

Family studies with genome-wide scanning using microsatellites are ongoing, and in the near future we will probably know which are the other DNA regions containing the susceptibility loci for APS. For the moment, we can say that the etiopathogenesis of APS has a strong genetic component, and

that this genetic predisposition is at least in part contributed by HLA alleles. The various studies performed indicate that the HLA alleles most frequently associated with APS are HLA-DRB1∗04 (DR4), DRB1∗07 (DR7), DRB1∗1302 (DR6), DRw53, DQA1∗0102, DQA1∗0201,DQA1∗0301, DQB1∗0302 (DQ8), and DQB1∗0604/5/6/7/9.

Studies on primary APS indicate that it is genetically distinct from SLE. In fact, although DR3 is the class II allele of greatest importance in SLE, this allele seems to be decreased in patients with the primary APS, where, by contrast, DR4, DR7, and DRw53 are the associated alleles. The picture is less clear if one looks at aPL. Of course, when these autoantibodies are found in patients with the primary APS, they show the same associations, but these HLA associations become less evident in SLE and disappear in other diseases, whether autoimmune or not. It seems that the association with DR4, DR7, DRw53, and DQB1∗0302 in SLE is only evident when aCL are found in patients with secondary APS.

Furthermore, the ethnic origins of patients also influence the pattern of HLA associations. For example, some alleles may appear because their higher prevalence in a given ethnic group, such as DRB1∗09 in Japanese populations.

In conclusion, immunogenetic studies suggest that APS is an entity distinct from SLE, even if it can appear in the course of this latter disease. The genetic predisposition to APS can be at least in part explained by an influence of certain HLA alleles. However, these alleles may only be apparent because of their linkage disequilibrium with an as yet unidentified primarily involved HLA locus, or they could act in cooperation with other genes, even residing outside the MHC. For this reason, the search for a more strongly associated polymorphism is actively pursued whenever new loci are identified in the HLA region.

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CHAPTER 7

Genetic Aspects of the Antiphospholipid Syndrome: Association with Clinical Manifestations

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1. Introduction

Antiphospholipid antibodies (aPL) are related to thrombosis in the antiphospholipid syndrome (APS) and thromboses are, together with obstetric complications, the main clinical manifestations of the APS. Numerous pathophysiological mechanisms have been suggested to explain thrombotic events, in both arterial and venous territories, in APS involving cellular effects, plasma coagulation regulatory proteins, and fibrinolysis. However, although there is a clear epidemiological association, not all aPL-positive individuals experience such complications. The heterogeneity of thrombotic manifestations in APS suggests that other additional factors may contribute to determine the ''prothrombotic profile'' in these patients: first, several characteristics of the aPL, such as the concentration, class/subclass, affinity, or charge, and several characteristics of the antigens, such as the concentration, size, location, or charge, may influence whether the aPL will act as prothrombotics in vivo (Roubey, 1996); second, oral contraception, pregnancy, surgery, trauma, smoking, immobilization, and other environmental causes can modify the thrombotic risk; and third, individual patient variability due to a predetermined genetic profile can modulate the effect of aPL on hemostasis. Thrombosis is supposed to result from the interaction of genetic characteristics with environmental or acquired factors, such as aPL, causing together the thrombus growth.

There are two levels of prothombotic genetic characteristics potentially related to the clinical expression of APS: the major alterations, consisting of deficiencies or polymorphisms clearly related to thrombosis (mainly related to venous thromboembolism), which are for this reason included in the usual thrombophilia test profiles; and a series of polymorphisms that have a little prothrombotic role on their own, but can significantly modify the effect of aPL on hemostasis.

2. Major alterations of thrombophilia

The main genetic thrombophilic defects include deficiencies of antithrombin, protein C and protein S, factor V Leiden, and the prothrombin G20210A mutation.

2.1. Antithrombin, protein C, and protein S deficiencies

Congenital deficiencies of antithrombin, protein C and protein S are very uncommon diseases and, for this reason, the number of patients with these deficiencies is too small to allow an accurate assessment of the associated risk of thrombosis in

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APS (Brouwer et al., 2004). By contrast, low levels of free protein S deficiency are seen in a high number of APS patients, suggesting an acquired origin like high levels of C4-binding protein. Although it is likely that these genetic defects may increase the risk of venous thromboembolism in patients with APS, only little is known about its possible interactions (Brouwer et al., 2004).

Anecdotically, a single patient suffering from recurrent thrombosis with type II plasminogen deficiency, a controversial thrombophilia factor deficiency, associated with APS and systemic lupus erythematosus (SLE) has been described (Iguchi et al., 2002). This case may be coincidental or it may be that the plasminogen deficiency has increased the thrombotic tendency of APS in this patient.

2.2. Factor V Leiden

The G1691A polymorphism in the factor V gene (factor V Leiden) is the most common hereditary risk factor for venous thrombosis in white individuals. Factor V Leiden prevalence in patients with familial thrombophilia is around 20% and up to 3% of the white population is a carrier of this polymorphism.

In early studies including few patients, some authors have identified factor V Leiden as a possible contributor to the occurrence of venous thrombosis associated with aPL (Bokarewa et al., 1995; Montaruli et al., 1996; Simantov et al., 1996) whereas others could not find a significant association between factor V Leiden and aPL in patients with thrombosis (Biousse et al., 1995; Davies et al., 1995; Dizon-Twonson et al., 1995). In a larger series, Fijnheer et al. (1996) found in 173 patients with SLE a frequency of factor V Leiden comparable to that seen in the normal Dutch population (5%), and that factor V Leiden was a risk factor for venous thrombosis (odds ratio (OR) = 4.9) but not for arterial thrombosis in SLE patients. The lupus anticoagulant was also a risk factor for both arterial thrombosis (OR = 7.1) and venous thrombosis (OR = 6.4). By multivariate analysis lupus anticoagulant and factor V Leiden appeared as independent risk factors and their risk for venous thrombosis was increased by a factor of 7 when lupus anticoagulants and factor V Leiden were both present (Fijnheer et al., 1996). In a similar manner, in a series of 152 lupus anticoagulant-positive patients Galli et al. (2000) demonstrated an association between factor V Leiden and venous thrombosis. Lupus anticoagulant and factor V Leiden were independent risk factors for venous thrombosis. No relationship was found between factor V Leiden and arterial thrombosis in these patients (Galli et al., 2000). These results were confirmed by Ames et al. (2001), that found in 49 aPL-positive patients a statistically significant higher prevalence of factor V Leiden in aPL thrombotic patients (14%) and in non-aPL thrombotic patients (18%) than in controls (4%), and also by Brouwer et al. (2004), who in a cohort of 144 consecutive patients with SLE identified that factor V Leiden in combination with aPL increased the venous thrombombolism risk of 30-fold as an independent risk factor. In another study of 147 aPL-positive patients (69 with previous thrombosis) who had anticardiolipin antibodies, 15% of patients with arterial thrombosis had factor V mutation compared with 3.5% of patients without thrombosis (OR = 4.9) (Chopra et al., 2002). However, in this study stepwise logistic regression analysis indicated that lupus anticoagulant, male sex, and hypertension were the strongest risk factors for developing thrombosis and that no additional risk was conferred by factor V Leiden. In a series of 120 white patients with SLE with or without aPL (Regéczy et al., 2000), factor V Leiden mutation and aPL were demonstrated to have an additive thrombogenic effect, providing higher predisposition to several vaso-occlusive disorders in SLE. Finally, Berkun and colleagues, in a cohort of 28 children with APS and thrombosis, found a high rate (45.5%) of inherited thrombophilic defects and their combinations, mainly due to the presence of factor V Leiden (Berkun et al., 2006). However, recurrent thromboses were not associated with hereditary thrombophilia.

Other authors, however, did not find an association between factor V Leiden and increased

thrombotic risk in APS. In a multicenter study in 75 patients with primary APS and 83 with SLE and aPL with or without thrombosis (Pablos et al., 1999), patients with venous thromboembolism or arterial thrombosis did not have a significantly increased frequency of factor V mutation compared with controls or patients with aPL without thrombosis. However, due to the sample size and a trend toward increased risk for thrombosis detected in patients with factor V Leiden, the authors cannot rule out synergy between both factor V Leiden and aPL (Pablos et al., 1999). In the same way, Torresan et al. (2000), in 30 Brazilian APS patients, and Forastiero et al. (2001) in a case–control study including 105 consecutive unselected white patients with aPL (69 having APS), found a similar prevalences of factor V Leiden in patients and in the control group. Moreover, cerebrovascular disease was not related to factor V Leiden in 44 patients with primary APS (Kalashnikova et al., 2005). In a recent study in 105 white SLE-patients (22 aPL-positive) (Sallai et al., 2007) the presence of factor V Leiden in addition to a positive aPL test only increased the relative risk of thrombosis slightly and did not reach the level of statistical significance. Factor V Leiden carried an increased risk of thrombosis within the aPL-negative patients (relative risk (RR) = 5.17), but in the aPL-positive patients this effect was statistically negligible (RR = 1.18). Globally, aPL was a more significant risk factor for the development of thrombosis than inherited thrombophilia causes (Sallai et al., 2007).

Two studies have demonstrated associations between familial aPL and factor V Leiden and reported its impact in thrombosis. In the first of them (Brenner et al., 1996), there were two young siblings who presented with recurrent severe thromboembolisms, familial APS, and were heterozygous for the factor V Leiden. In the second (Schütt et al., 1998), autoimmune thrombocytopenia was documented in 6 out of 11 family member patients, in three cases associated with primary APS and with the presence of factor V Leiden. Only these three patients had thromboembolic disease, while the other thrombocytopenic family members showed no thrombotic manifestations.

From a pathogenetic perspective factor V Leiden and aPL-positive individuals may have in common increased thrombin generation related to impaired protein C function (activated protein C resistance). In the former case activated protein C cannot exert its anticoagulant properties on mutated factor V; in the latter, protein C function is directly impaired by aPL or by the acquired deficiency of free protein S. Interestingly, aPL-related activated protein C resistance does not appear in many cases to be related to a mutation in the coagulation factor V gene (acquired activated protein C resistance) (Bokarewa et al., 1995; Muñoz-Rodríguez et al., 2002), but acquired activated protein C resistance, associated or not with anti-protein S antibodies (Nojima et al., 2002), is related to thrombosis and pregnancy losses in APS (Muñoz-Rodríguez et al., 2002).

In conclusion, factor V Leiden seems to have a milder effect on the development of thrombosis in APS compared with that seen in the general population due to the strong effect of aPL, but factor V Leiden may in several patients increase the thrombogenic effect of aPL.

2.3. Prothrombin G20210A mutation

The G20210A variation in the gene coding for factor II (prothrombin) is also a common polymorphism associated with venous thromboembolism. The prevalence of the prothrombin 20210A allele in unselected patients with a thrombotic event is about 15% and its prevalence in normal white populations ranges between 2% and 4%.

The initial studies did not show an increased risk of thrombosis related to the G20210A polymorphism in the prothrombin gene in APS patients of white (Bentolila et al., 1997; Bertolaccini et al., 1998) or Mexican mestizo origin (Ruíz-Argüelles et al., 1999). However, from the first case of SLE-associated APS in a young female homozygous for the 20210A allele in the prothrombin

gene who developed venous thrombosis while taking oral contraceptives (Sivera et al., 2000) several subsequent studies have demonstrated the association between the prothrombin G20210A polymorphism and thrombosis in APS patients. Torresan et al. (2000) found in 30 Brazilian patients with APS and thrombosis a higher prevalence of the allele 20210A of the prothrombin gene in APS patients when compared with controls (5% vs. 0.7%). Similarly, Forastiero et al. (2001) found in 105 white consecutive unselected patients with aPL grouped as having APS ($n = 69$) and not having APS ($n = 36$) that the 20210A allele was significantly more frequent in APS patients than in normal controls (8.7% versus 2%). Brouwer et al. (2004) in a cohort of 144 consecutive patients with SLE, found that the 20210A allele of the prothrombin gene was an independent risk factor for venous thromboembolism that when presented together with aPL increased the risk 30-fold.

Other studies, however, have shown no relationship between prothrombin G20210A polymorphism and thrombosis in APS. In the study of Galli et al. (2000) the prevalence of the G20210A polymorphism was evaluated in 145 white aPL-positive patients and they found no association between the 20210A allele and venous thrombosis. Similarly, in 157 aPL-positive patients (69 with previous thrombosis), the G20210A polymorphism was not associated with thrombosis (Chopra et al., 2002). Finally, in a recent study (Sallai et al., 2007) in 105 SLE patients, the prothrombin G20210A polymorphism was not associated with thrombosis risk.

3. Minor alterations of thrombophilia

Several polymorphisms have been postulated to have the potential to modify the thrombotic risk in aPL-positive patients (Table 1). Some of these polymorphisms affect proteins directly related to aPL, others are related to normal hemostasis components, and, finally, others are related to immune or inflammatory pathways.

Table 1
Genetic thrombophilia polymorphisms evaluated for its eventual association with clinical manifestations of APS

- Major alterations of thrombophilia
  - Antithrombin, protein C and protein S deficiencies
  - Factor V Leiden
  - Prothrombin G20210 A mutation
- Minor alterations of thrombophilia.
  - Polymorphisms in $\beta_2$glycoprotein I
  - Polymorphisms in platelet Fcγ receptor IIA
  - Polymorphisms in platelet glycoproteins
  - Thermolabile variant of the methylenetetrahydrofolate reductase
  - Type-1 plasminogen activator inhibitor polymorphism
  - Tissue factor pathway inhibitor polymorphisms
  - Factor XIII polymorphisms
  - P-selectin polymorphisms
  - P-selectin glycoprotein ligand-1 polymorphisms
  - CD40 ligand polymorphisms
  - Tumor necrosis factor α polymorphisms
  - Angiotensin-converting enzyme polymorphisms
  - Mannose-binding lectin polymorphisms
  - Toll-like receptor 4 polymorphisms

3.1. Polymorphisms in β_2 glycoprotein I

Several β_2 glycoprotein I (β_2GPI) gene polymorphisms have been studied in its association with clinical manifestations of the APS and with the development of antibodies against β_2GPI (anti-β_2GPI). The Val247Leu β_2GPI gene polymorphism has been the most studied of these polymorphisms in APS patients.

In relation to the development of anti-β_2GPI, in 88 white patients with APS (57 with primary APS and 31 with APS secondary to SLE) (Atsumi et al., 1999) the Val247 allele of the Val247Leu β_2GPI gene polymorphism was significantly more frequent in primary APS patients with anti-β_2GPI than in controls (OR = 2.5) or in primary APS patients without anti-β_2GPI (OR = 2.9), but no relationship was found in the secondary APS group between the Val247Leu β_2GPI poliymorphism and anti-β_2GPI. These results were confirmed in a series of 33 Mexican patients with primary APS (Prieto et al., 2003). In this study anti-β_2GPI-positive patients had significantly higher frequencies of the Val/Val genotype (0.37 vs. 0.13)

and Val247 allele (0.58 vs. 0.39) than the control subjects and the anti-β_2GPI-negative patients. Similar results were found in Asian patients (Hirose et al., 1999) in whom Val247, especially in the homozygous state, was significantly associated with the presence of anti-β_2GPI. However, in this study (Hirose et al., 1999) no significant differences in allele or genotype frequencies of the Val247Leu β_2GPI polymorphism were seen in white or African-American patients in comparison with appropriate controls. In addition, in 222 white women with SLE with and without APS the Val247Leu β_2GPI polymorphism was not related to the presence of anti-β_2GPI (Kamboh et al., 1999).

In reference to the relationship to the Val247Leu β_2GPI polymorphism and thrombosis in APS patients, in a series of 20 Mexican patients with primary APS both the Val/Val genotype (0.54 vs. 0.00) and Val247 allele frequencies (0.68 vs. 0.36) were significantly higher in patients with arterial thrombosis than in patients without arterial thrombosis (Prieto et al., 2003). No association of the β_2GPI gene polymorphism with thrombocytopenia, venous thrombosis, livedo reticularis, recurrent fetal loss, or hemolytic anemia was found (Prieto et al., 2003). However, it is not clear if the Val/Val genotype itself confers a higher risk of developing arterial thrombosis or if this clinical feature is determined only for the higher frequency of anti-β_2GPI.

Other β_2GPI polymorphisms studied in APS include Ser88Asn, Cys306Gly, and Trp316Ser. In 143 patients with SLE and/or APS no significant association was found between either polymorphisms Cys306Gly or Trp316Ser with aPL or anti-β_2GPI, but there was a trend favoring the development of thrombosis in heterozygous individuals with Trp316Ser polymorphism of β_2GPI, especially in patients with SLE, in whom it was independent of the presence of aPL (Gushiken et al., 1999). In a study in 222 white women with SLE, none of the Ser88Asn, Cys306Gly, and Trp316Ser polymorphisms were associated with the occurrence of anti-β_2GPI (Kamboh et al., 1999), but the frequency of Ser316 was significantly lower in aPL-positive patients than in the aPL-negative (3.1% vs. 12.1%).

3.2. Polymorphisms in platelet Fcγ receptor IIA

Platelet Fcγ receptor IIA (FcγRIIA; CD32) molecules are essential for the effect of aPL against β_2GPI by causing platelet activation, thromboxane A_2 generation, and granule release after their binding to the Fc fragments of aPL. Human FcγRIIA reacts best with IgG subclasses 1 and 3, but weakly with subclass 2, which includes the majority of the anti-β_2GPI seen in autoimmune patients (Arvieux et al., 1994). The His131 allele of the His131Arg polymorphism of the FcγRIIA gene reacts much more efficiently than the Arg131 allele to IgG subclass 2. As it has been reported in heparin-induced thrombocytopenia (Carlsson et al., 1998), Atsumi et al. (1998) tested whether patients with the His131 allele of the FcγRIIA polymorphism may be at greater risk of developing thrombosis by this platelet activation mechanism. They studied 100 white patients with aPL and none of the clinical manifestations of primary APS (arterial or venous thrombosis, recurrent pregnancy loss, and thrombocytopenia) was significantly correlated with the His131Arg FcγRIIA polymorphism. However, in a more recent meta-analysis (Karassa et al., 2003) a significant increase in Arg131 homozygosity was found in APS patients and the authors suggested a complex genetic background underlying the relationship between the FcγRIIA Arg131His polymorphism and the APS as a composite of two different and opposing influences with regard to susceptibility. Unfortunately, the number of APS patients with specific clinical manifestations was too small to reliably assess the effect of the FcγRIIA polymorphism on the risk of vascular thromboses or other APS-related features. More recently (Schallmoser et al., 2005) the Arg131His FcγRIIA polymorphism was evaluated in 73 aPL-positive patients (47 with thrombosis) and an increased frequency of heterozygous patients was associated with thrombosis (OR = 6.76). In this study heterozygosity, rather than Arg131 homozygosity, was linked to the clinical manifestations of APS. The authors explained these data by the dual function of the FcγRIIA, namely binding

of antibodies to platelets and thereby their activation, and, on the other hand, clearance of antibody-coated platelets by the phagocyte system.

3.3. Polymorphisms in platelet glycoproteins

The interaction of aPL with platelets by increasing the adhesion of platelets to the subendothelium is one of the mechanisms proposed for increased arterial thrombotic risk. The main platelet receptors are GPIb-alpha, GPIa/IIa and GPIIb/IIIa. Genetic variability affecting platelet receptors involved in adhesion processes has been related to increased risk of arterial thrombosis in non-APS patients (Nurden, 1995).

Tolusso et al. (2003) analyzed the P1A1/2 polymorphism of GPIIb/IIIa in patients with SLE in relation to ischemic manifestations and only found a relationship with Raynaud's phenomenon. More recently, we have studied three polymorphisms in GPIb-α, GPIa/IIa and GPIIb/IIIa in a cohort of 131 white APS patients (Jiménez et al., 2008): in GPIb-alpha the length polymorphism from a variable number of tandem repeats (VNTR) (alleles designated A, B, C, and D), in GPIa/IIa the C807T polymorphism of the Ia subunit related to receptor expression (T allele carriers with increased receptor density), and in GPIIb/IIIa the P1A1/2 polymorphism in the IIIa subunit (the prothrombotic allele is P1A2). In this study, we found the homozygous 807T allele of GPIa/IIa more frequently in patients with APS who had arterial thrombosis (OR = 3.59), suggesting that this polymorphism may be an additional risk factor for arterial thrombosis in APS patients. There was no association between polymorphisms in GPIb-alpha and GPIIb/IIIa and arterial thrombosis. Interestingly, we also found a gene–gene interaction between GPIa/IIa and GPIIb/IIIa for arterial thrombosis. The coexistence of at least one 807T allele in GPIa/IIa together with at least one P1A2 allele in GPIIb/IIIa significantly increased the risk for arterial thrombosis in our series of APS patients (OR = 4.84). This gene–gene interaction may be explained by a functional interrelation between these two platelet glycoproteins recently demonstrated in experimental in vitro studies, showing that the activation of GPIIb/IIIa is a prerequisite for the activation of the GPIa/IIa (Van de Walle et al., 2007). We did not find any relationship between the polymorphisms analyzed, either individually or in combination, with the presence of venous thrombosis or pregnancy morbidity in patients with APS. In the future, the study of new gene–gene and gene–environment interactions would identify specific combinations that are associated with the clinical manifestations in APS.

3.4. Thermolabile variant of methylenetetrahydrofolate reductase

The C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism (thermolabile variant 677TT) has been shown to exert a potential effect on plasma homocysteine levels. At the present, this polymorphism is not considered per se to be a risk factor for thrombosis (Bertina, 2001) and is recommended to exclude C677T MTHFR polymorphism from the analysis of multiple thrombophilic genotypes.

In a series of 152 aPL-positive patients, Galli et al. (2000) did not find association between the C677T MTHFR polymorphism alone or in combination with either factor V Leiden or G20210A prothrombin polymorphisms and thrombosis. Similar results were obtained by Torresan et al. (2000) in 30 patients with APS in whom no significant variation was found between the patient group and the controls regarding the prevalence of homozygotes for the mutated 677T allele (2.5 vs. 5.4%), and by Forastiero et al. (2001) in 105 aPL-positive patients in whom the frequencies of the C677T MTHFR alleles were not different either between the aPL groups and normal controls or between APS and non-APS groups. In addition, cerebrovascular disease was not related to homozygous or heterozygous MTHFR in 44 primary APS patients (Kalashnikova et al., 2005). Finally, Ames et al. (2001) in 49 aPL-positive subjects also observed that the C677T MTHFR polymorphism

was not related to venous thrombosis, but the homozygous 677TT patients had lower mean age at first event and suffered an increased average number of events per person.

3.5. Type-1 plasminogen activator inhibitor polymorphism

The possible contribution of fibrinolysis to the development of thrombosis in the APS is very stimulating due to the interrelation between the fibrinolysis activation pathways and the inflammatory events present in autoimmune diseases. Several studies have suggested that in patients with APS fibrinolysis may be impaired, mainly due to an excess of type 1 plasminogen activator inhibitor (PAI-1) (Tsakiris et al., 1989) but other have found discordant results (Mackworth-Young et al., 1995). Plasma PAI-1 levels are related to a single base-pair guanosine deletion/insertion polymorphism (4G/5G) located in the promoter region of the PAI-1 gene, because the 4G allele is associated with enhanced PAI-1 expression due to the differential binding to the polymorphic site of nuclear proteins involved in the activation and the inhibition of gene transcription.

To test the hypothesis that some of the discrepancies in PAI-1 predictive value in thrombosis in APS patients are partially related to a predetermined genetic substrate, we studied the 4G/5G polymorphism of the PAI-1 gene in a series of 70 white patients with primary APS and 104 patients with SLE (40 with secondary APS) (Tàssies et al., 2000). We found higher prevalence of the 4G allele in APS patients with thrombosis in comparison with APS patients without thrombosis (0.57 vs. 0.39, OR = 2.83 for thrombosis in APS patients with at least one 4G allele). These differences were attributable to the higher 4G prevalence found in patients with arterial thrombosis (0.64 vs. 0.43; OR = 5.96 for arterial thrombosis in APS patients with at least one 4G allele), while patients with venous thrombosis had an allele distribution similar to that found in patients without venous thrombosis. Although PAI-1 antigen levels and PAI-1 activity did not differ significantly among patient groups or controls, the influence of the genotype of the PAI-1 gene on the local production of PAI-1 at the site of vessel injury may be important in thrombus formation and not be reflected in plasma PAI-1 levels. However, in 77 Japanese and 82 British patients with aPL (Yasuda et al., 2002) no correlation was found between the 4G/5G PAI-1 polymorphism (and also the Alu-repeat insertion/deletion polymorphism of the tissue-type plasminogen activator) and clinical symptoms of APS (arterial or venous thrombosis, miscarriage). In their study in 105 aPL-positive individuals, Forastiero et al. (2001) did not find a statistically significant relationship between 4G/5G PAI-1 genotype and thrombosis, but found a trend towards a higher frequency of 4G/4G PAI-1 in aPL patients with arterial thrombosis versus those with venous thrombosis. They also found that a statistically significant higher proportion of aPL patients with thrombosis have the 20210A prothrombin allele combined with the 4G/4G PAI-1 genotype compared with patients without thrombosis. Finally, in a meta-analysis (Tsantes et al., 2007) the presence of the 4G allele might significantly increase the thrombotic risk in patients with other causes of thrombophilia and to a lesser degree in cases without known risk factors.

3.6. Tissue factor pathway inhibitor polymorphisms

Increased tissue factor activity on circulating blood is an important mechanism of hypercoagulability in APS and tissue factor pathway inhibitor (TFPI) is its main physiological inhibitor.

In a recent study (Lincz et al., 2007) TFPI activity and the frequency of common TFPI polymorphisms, −33T→C, −399C→T and −287T→C, was evaluated in 24 APS patients and also in 44 patients with factor V Leiden and thrombosis. They found that only APS patients with a history of venous thrombosis had TFPI activity levels significantly different from healthy controls, and this was significantly associated with inheritance of the TFPI −33C allele (higher TFPI values in TC/CC genotypes and lower in TT). The TFPI −33C and the TFPI −399T alleles were associated

with venous thromboembolism in a group composed of APS patients and factor V Leiden patients. The fact that IgG anti-β_2GPI can accelerate thrombin generation in the presence of TFPI may contribute to explain these data (Lean et al., 2006).

3.7. Factor XIII polymorphisms

Coagulation factor XIII plays a major role in the final stage of blood coagulation. Factor XIII catalyzes crosslinking between fibrin molecules. Factor XIII polymorphic sites have been described at Va134Leu, Pro564Leu, Val650Ile, Glu651Gln, and Tyr204Phe. The Val34Leu polymorphism has been reported to be protective against both arterial and venous thrombosis (Elbaz et al., 2000).

Diz-Kucukkaya et al. (2004) investigated whether the factor XIII Val34Leu polymorphism is protective against the development of thrombosis in APS patients. They evaluated 60 APS patients with thrombosis and 126 healthy controls and they found that Leu34 allele had no protective effect on the development of thrombosis in APS. Recently, we have expanded the study including the effect of fibrinogen levels because it has been described that in vitro fibrin structure may be modified by interactions between fibrinogen concentration and factor XIII Val34Leu polymorphism (Lim et al., 2003). In 172 consecutive patients with aPL (83 with APS) we did not find statistically significant differences in factor XIII Leu34 allele frequency between APS patients with thrombosis and patients without thrombosis, but when we stratified the patients according to fibrinogen levels, we observed a statistically significant difference showing that factor XIII Leu34 allele seems to have a protective effect in the development of thrombosis in patients with fibrinogen in the upper tertile but not in patients with lower fibrinogen levels (De la Red et al., 2009).

3.8. P-Selectin polymorphisms

P-Selectin mediates the attachment and rolling of leukocytes on activated endothelial cells, and is involved in the recruitment of leukocytes and microparticles to thrombi.

The Thr715Pro P-selectin polymorphism has been associated with myocardial infaction (Herrmann et al., 1998).

Recently, in a study (Bugert et al., 2007) in 107 aPL-positive patients (74 with thrombosis) the P-selectin Pro715 allele was more frequent in patients with venous thrombosis (OR = 3.2) but no patient with arterial thrombosis had this allele (OR = 0.1). The authors, taking together their data with similar findings described for patients suffering from myocardial infarction (Herrmann et al., 1998), suggest that the Pro715 allele may strengthen platelet adhesion to venous but not to arterial endothelium.

3.9. P-Selectin glycoprotein ligand 1 polymorphisms

P-Selectin glycoprotein ligand 1 (PSGL-1) is the major leukocyte and microparticles counter-receptor for P-selectin. P-selectin/PSGL-1 interaction is crucial in inflammation and thrombosis. A variable number of tandem repeats (VNTR) polymorphism has been described in PSGL-1 with three alleles A, B, and C (allele A being the longer). The VNTR PSGL-1 polymorphism has been evaluated in 90 aPL-positive patients (Diz-Kucukkaya et al., 2007) and the AB-carrying APS patients had increased risk for both arterial and venous thrombosis. The presence of the BB genotype, however, was not associated with thrombotic risk. However, Bugert et al. (2007) in 107 aPL-positive patients found no relationship between the VNTR PSGL-1 polymorphism, nor two other PSGL-1 polymorphisms (Met62Ile and Ser273Phe), and venous thrombosis.

3.10. CD40 ligand (CD154) polymorphisms

Increased levels of soluble CD154 have been described in various inflammatory disorders, particularly SLE. A polymorphic CA repeat

sequence has been identified in the 3′-untranslated region of the CD154 gene. The larger alleles of this CA repeat are more frequent in SLE patients and are also associated with a prolonged protein expression on T lymphocytes.

In a recent study in 107 aPL-positive patients, Bugert et al. (2007) found than the CA repeat polymorphism in the 3′-untranslated region of CD154 was associated with the development of arterial thrombosis (applying the dominant model and considering CD154 genotype exclusively containing alleles with 24 CA repeats, OR = 4.04) but not with venous thrombosis.

3.11. Tumor necrosis factor-alpha polymorphisms

Bertolaccini et al. (2001) explored in 83 white patients with APS the possible involvement of the proinflammatory and prothrombotic cytokine tumor necrosis factor-alpha (TNF-α) in APS, and observed that the presence of the −238∗A genotype in the promoter region of the TNF-α gene was more frequent in APS patients with arterial thrombosis and pregnancy loss than in controls (OR = 3.7).

3.12. Angiotensin-converting enzyme polymorphisms

Evaluating the reported association between the D allele of the insertion (I)/deletion (D) polymorphism in the angiotensin-converting enzyme (ACE) gene and the occurrence of arterial thrombosis in coronary heart disease and stroke, Lewis et al. (2000) studied in 93 patients with APS whether this polymorphism could be an additional risk factor for arterial thrombosis. The distribution of the alleles was not significantly different between the patients with a history of arterial thrombosis and those without, although an unexpected skewing from DD to II was seen patients older than 45 years in association with arterial thrombosis.

3.13. Mannose-binding lectin polymorphisms

Innate immunity is the first-line defense against pathogens. Among the components of innate immunity mannose-binding lectin (MBL) and toll-like receptor 4 polymorphisms have been related to APS clinical manifestations.

MBL is a liver-derived serum protein that binds to sugars on the surface of pathogenic microorganisms and triggers complement. Serum levels of MBL are associated with MBL gene polymorphisms. In 91 white patients with SLE MBL variant alleles were evaluated and a statistically significant association has been found between the deficient homozygous 0/0 MBL genotype and the development of arterial thrombosis (OR = 5.8), but not venous thrombosis, mainly due to the strong association between this genotype and myocardial infarction (Øhlenschlaeger et al., 2004). However, MBL polymorphisms were not specifically evaluated in the APS subgroup. We have studied MBL polymorphisms in a series of 114 white SLE patients (Font et al., 2007) and found that MBL-low genotypes showed a closer association with venous rather than arterial thrombosis. This fact probably is due to the different MBL alleles analyzed (0/XA and XA/XA were also included as deficient alleles) and/or to the varying prevalence of thrombotic events. In addition, in 53 patients with SLE, Seelen et al. (2005) reported that the presence of aCL was significantly associated with the variant alleles of MBL gene polymorphisms and they hypothesized that an enhanced production of autoantibodies may be related to disturbed clearance of apoptotic material due to impaired MBL function. Additional contradictory results have been found in non-APS patients. In several different ethnic origin SLE patients Calvo-Alén et al. (2006) found no differences in arterial thrombosis in patients homozygous for MBL-deficient alleles compared with the others. Similar results were seen within ethnic groups, except for white patients in whom a statistically significant higher frequency of MBL-deficient alleles was found in those with cerebrovascular events. In a Japanese population Takahashi et al.

(2005) did not find a relationship between MBL alleles and the risk of arterial thrombosis. These results point out the need to bear in mind the importance of the differences in the genetic substrate among the ethnic group when evaluating the influence of genetic polymorphisms in APS clinical expression.

3.14. Toll-like receptor 4 polymorphisms

Toll-like receptor 4 (TLR4) belongs to the family of transmembrane receptors whose activation leads to induction of various genes and production of proinflammatory cytokines. Polymorphisms within TLR4 genes result in an altered susceptibility to infectious or inflammatory diseases.

In 110 white patients with APS with arterial and/or venous thrombosis Pierangeli et al. (2007) evaluated whether the two co-segregating TLR4 polymorphisms Asp299Gly and Thr399Ile are involved in aPL-mediated thrombosis. They also studied endothelial cell activation in vivo in a mouse model. This study showed that the frequency of TLR4 Gly299 and Ile399 alleles in APS patients was significantly reduced in comparison to healthy controls. These facts suggest that a strong inflammatory response may favor the susceptibility to thrombosis in the presence of aPL.

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CHAPTER 8

Systemic Manifestations of the Antiphospholipid Syndrome

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1. Introduction

Single vessel involvement or multiple vascular occlusions may give rise to a wide variety of presentations in the antiphospholipid syndrome (APS). Any combination of vascular occlusive events may occur in the same individual and the time interval between them also varies considerably from weeks to months or even years. The study of 1000 European APS patients (''Euro-Phospholipid project'') has provided accurate information on the prevalence of the majority of clinical manifestations of this syndrome (Table 1) (Cervera et al., 2002).

2. Large vessel manifestations

2.1. Venous occlusions

Venous occlusions, particularly affecting the deep veins of the lower limbs (i.e. deep vein thrombosis (DVT)) are the commonest clinical manifestation of the APS (Cervera et al., 2002). Less commonly, a superficial thrombophlebitis may be seen. Chronic venous stasis and malleolar ulceration may result after episodes of DVT. On occasion, other large veins may be thrombosed (e.g. subclavian, external jugular, ileo-femoral or even the vena cava itself (both inferior and superior)).

Venous occlusions occurring at sites of venous access, such as indwelling venous catheters, are not uncommon in patients with the APS. Particular care should be taken by APS patients to exercise frequently in situations requiring prolonged immobilization, such as prolonged bed rest, particularly following surgical procedures, long air journeys, or the wearing of plaster casts on limbs because of fractures.

2.2. Arterial occlusions

The thoracic branches of aorta may be affected and an ''aortic arch syndrome'' with absent brachial pulse has been documented (Asherson et al., 1985). Occlusion of the abdominal aorta itself has also been reported on several occasions (McGee et al., 1992). Narrowing of the ileo-femoral arteries with resultant claudication and eventual gangrene of the extremities has also been documented on many occasions (Setoguchi et al., 1997).

3. Neurologic manifestations

3.1. Cerebral infarctions

These manifestations are second only to DVT in frequency in the APS (Cervera et al., 2002). They are often multiple, recurrent, and, most commonly, affect the territory of the middle cerebral artery, with lesions occurring predominantly in the frontal and parietal lobes. After a first ischemic

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Table 1
Cumulative clinical features during the evolution of the disease in 1000 patients with APS

Manifestations	No.	(%)
Peripheral thrombosis		
Deep vein thrombosis	389	(38.9)
Superficial thrombophebilitis in legs	117	(11.7)
Arterial thrombosis in legs	43	(4.3)
Venous thrombosis in arms	34	(3.4)
Arterial thrombosis in arms	27	(2.7)
Subclavian vein thrombosis	18	(1.8)
Jugular vein thrombosis	9	(0.9)
Neurologic manifestations		
Migraine	202	(20.2)
Stroke	198	(19.8)
Transient ischemic attack	111	(11.1)
Epilepsy	70	(7)
Multi-infarct dementia	25	(2.5)
Chorea	13	(1.3)
Acute encephalopathy	11	(1.1)
Transient amnesia	7	(0.7)
Cerebral venous thrombosis	7	(0.7)
Cerebellar ataxia	7	(0.7)
Transverse myelopathy	4	(0.4)
Hemiballismus	3	(0.3)
Pulmonary manifestations		
Pulmonary embolism	141	(14.1)
Pulmonary hypertension	22	(2.2)
Pulmonary microthrombosis	15	(1.5)
Fibrosant alveolitis	12	(1.2)
Other (adult respiratory distress syndrome, pulmonary hemorrhage, pulmonary artery thrombosis)	7	(0.7)
Cardiac manifestations		
Valve thickening/dysfunction	116	(11.6)
Myocardial infarction	55	(5.5)
Angina	27	(2.7)
Myocardiopathy	29	(2.9)
Vegetations	27	(2.7)
Coronary bypass rethrombosis	11	(1.1)
Intracardiac thrombus	4	(0.4)
Intraabdominal manifestations		
Renal manifestations (glomerular thrombosis, renal infarction, renal artery thrombosis, renal vein thrombosis)	27	(2.7)
Gastrointestinal manifestations (esophageal or mesenteric ischemia)	15	(1.5)
Splenic infaction	11	(1.1)
Pancreatic infarction	5	(0.5)
Addison's syndrome	4	(0.4)
Hepatic manifestations (Budd–Chiari syndrome, small hepatic vein thrombosis)	7	(0.7)
Cutaneous manifestations		
Livedo reticularis	241	(24.1)
Ulcers	55	(5.5)
Pseudovasculitic lesions	39	(3.9)
Digital gangrene	33	(3.3)
Cutaneous necrosis	21	(2.1)
Splinter hemorrhages	7	(0.7)
Osteo-articular manifestations		
Arthralgia	387	(38.7)
Arthritis	271	(27.1)
Avascular necrosis of bone	24	(2.4)
Ophthalmologic manifestations		
Amaurosis fugax	54	(5.4)
Retinal artery thrombosis	15	(1.5)
Retinal vein thrombosis	9	(0.9)
Optic neuropathy	10	(1)
Ear, nose, and throat manifestations		
Nasal septum perforation	8	(0.8)
Hematological manifestations		
Thrombocytopenia ($<100\ 000/\mu L$)	296	(29.6)
Hemolytic anemia	97	(9.7)

stroke, the presence of anticardiolipin antibodies (aCL) has been shown to be associated with an increased risk of recurrence of stroke over 2 years as well as of other thromboembolic events and death (Stern and Brey, 1994).

The association between livedo reticularis and ischemic stroke, accompanied on occasion by hypertension, has been known as Sneddon's syndrome. It is more frequent in women, is usually diagnosed in the fourth or fifth decade, and there is a familial clustering in some patients. It can also be a manifestation of the primary APS (Asherson and Cervera, 1993a).

3.2. Transient ischemic attacks

Transient cerebrovascular ischemia resulting in amaurosis fugax, transient paresthesias, and motor weakness, have all been described in patients with APS.

3.3. Multi-infarct dementia

Several patients with multi-infarct dementia and antiphospholipid antibodies (aPL) have been documented (Gómez-Puerta et al., 2005). The clinical manifestations of dementia associated with the APS are no different to those encountered in patients with vascular dementia of any other cause.

3.4. Acute ischemic encephalopathy

These patients are acutely ill, confused, and obtunded, with an asymmetrical quadriparesis, hyperreflexia, and bilateral extensor plantar responses. Seizures may also occur. Small cortical hypodensities have been discernible on magnetic resonance imaging (MRI) scanning in several patients (Briley et al., 1989). With the unravelling of the catastrophic APS, it seems likely that cerebral thrombotic microangiopathy, predominant in that condition, is the basis of acute ischemic encephalopathy, a common accompaniment of the catastrophic APS itself (Bucciarelli et al., 2006).

3.5. Cerebral venous and dural sinus thrombosis

Cerebral venous sinus thrombosis (CVST) and dural sinus thrombosis have a diverse spectrum of clinical manifestations, the commonest being headache, accompanied by papilledema, nausea, vomiting, and visual field loss. CVST is one of the causes of the syndrome referred to as ''pseudotumor cerebri'' (benign intracranial hypertension), many cases of which are idiopathic and related to disturbed cerebrospinal fluid dynamics. Several cases of an association between CVST and aPL have been reported (Levine et al., 1987).

3.6. Psychosis

Several APS patients have been recorded in whom psychosis precedes by many years the occurrence of thrombotic symptoms (Jurtz and Muller, 1994). Increased aPL have been documented in schizophrenic patients (Firer et al., 1994) and in major depression (Maes et al., 1993). Their role in this group of conditions is undetermined at this time.

3.7. Cognitive defects

Cognitive defects, including behavioral and affective disturbances, are not uncommon in patients with systemic lupus erythematosus (SLE) and are usually ascribed to a ''lupus cerebritis.'' Work in animal models has related neurologic and behavioral deficits to be effects of the aPL. On immunofluorescence staining, immunoglobulin deposits have been observed in the vessel walls of brain derived from these animals (Ziporen et al., 1996). A few patients with APS who presented with rapidly progressive change in mental status, confusion, memory disturbance, and emotional lability have also been reported (Mikdashi et al., 1996). Transient global amnesia, a syndrome of sudden unexplained short-term memory loss associated often with stereotyped behavior, has been linked to aPL in one patient (Montalbán et al., 1989).

3.8. Pseudomultiple sclerosis

Several young patients who had fluctuating and recurrent neurologic events with focal and visual neurologic symptoms have been published. High signal lesions in the periventricular white matter on T2-weighted MRI resembled multiple sclerosis (Cuadrado et al., 2000).

3.9. Migraine and migranous stroke

Headaches, often non-migrainous in type, may precede or accompany transient ischemic attacks or strokes in aPL-positive patients. Migraine is, however, common in SLE. Although many investigators have commented on an association

between true migraine and the aPL (Hogan et al., 1988), several studies have not borne out any association (Montalbán et al., 1992).

3.10. Epilepsy

Epileptiform seizures are common in SLE and may precede the appearance of other serological or clinical evidence of the disease by many years. Herranz et al. (1994) studied 221 unselected patients with SLE, of whom 21 suffered from epileptic seizures not attributable to any cause other than SLE. Lupus anticoagulant was detected in 43.8% of the epileptic patients and in 20.8% of controls ($p = 0.057$). A statistically significant association was found between moderate-to-high titers of IgG aCL and the presence of seizures ($p = 0.02$).

3.11. Movement disorders

3.11.1. Chorea

This is an infrequent clinical manifestation of SLE (occurring in less than 4% of cases), that has been strongly linked to the presence of aPL. It does not differ from chorea encountered with rheumatic fever (Sydenhan's) or the inherited form (Huntington's). In a review of 50 cases, the authors found that 96% were females and that the mean age was 23 years. One episode of chorea was seen in 66% of the patients, while in 34% it was recurrent. Oral contraceptive-induced chorea, chorea gravidarium, and postpartum chorea occurred in 2–6% of patients. It was seen bilaterally or unilaterally; occasionally commenced on one side, to reappear on the other side within weeks to months. Computed tomography (CT) scanning was usually normal but infarcts outside the basal ganglia themselves were occasionally seen. MRI findings were only reported in 13 of the 50 cases and infarcts in the caudate nuclei were only seen in 3 (Cervera et al., 1997).

3.11.2. Hemiballismus

This rare movement disorder in an aCL-positive patient has also been recorded (Tam et al., 1995).

3.11.3. Cerebellar ataxia

This may also unusually be related to the presence of aPL (Singh et al., 1988).

3.12. Spinal syndromes

3.12.1. Transverse myelopathy

This is uncommon in SLE (occurs in less than 1% of patients) and is generally associated with a poor prognosis. Presentation is usually acute, with paresthesia in the legs, ascending to the thorax within 24–48 hours. Paraplegia, back pain, and loss sphincter control may follow. Several papers have stressed the occurrence of transverse myelitis with the presence of the aPL (Lavalle et al., 1990).

3.12.2. Guillain–Barré syndrome

Several APS patients with this complication have been documented. It has been suggested that aCL of the IgA isotype are associated with peak disease activity (Harris et al., 1983).

3.12.3. Anterior spinal artery syndrome

Sparing of the posterior columns occurs in this condition, with the patient presenting with a flaccid paraplegia, sphincter disturbances, and dissociated sensory impairment. One case with positive aCL has been documented (Harris et al., 1983).

3.13. Ophthalmic complications

Small vessel occlusions affecting the choroid, retina, and optic nerve result in ischemia and even infarctions. Neovascularization leads to secondary vitreous hemorrhage, traction retinal detachments or glaucoma. Several reports have estimated retinal vascular occlusions in 8–12% of patients with aPL. Optic neuropathy (acute retrobulbar optic neuritis, ischemic optic atrophy, and progressive optic atrophy) has also been linked to the presence of the aPL (Montehermoso et al., 1999).

4. Cardiac manifestations

4.1. Myocardial infarctions

Myocardial infarction (MI) in SLE is usually due to accelerated atherosclerosis or vasculitis but, since the discovery of the aPL, it has become evident that MI is not an uncommon accompaniment of the APS (Cervera, 2004). Conversely, reports of aPL in patients with MI have yielded conflicting results. While it has been reported that aCL are common in young post-infarction patients and should be regarded as markers for recurrent cardiovascular events (Hamsten et al., 1986), other investigators could not confirm this finding (Sletnes et al., 1992).

4.2. Coronary bypass graft and angioplasty occlusions

Elevated aCL levels in patients who developed late bypass vein graft occlusions have been detected (Morton et al., 1986). Another study (Eber et al., 1992) reported increased IgA aCL levels in men with coronary artery disease treated with percutaneous transluminal coronary angioplasty who restenosed.

4.3. Cardiomyopathy

Multiple small vascular occlusions (thrombotic microvasculopathy) are responsible for both acute and chronic cardiomyopathy seen in patients with aPL, the clinical picture being dependent on the rapidity of the process. Acute cardiac collapse (often together with respiratory decompensation) is frequent in patients with the catastrophic APS, and is one of the most common causes of death in this group of patients (Bucciarelli et al., 2006).

4.4. Valvular disease

4.4.1. Valve thickening

Valve thickening, resulting in valve dysfunction and incompetence, is common in the APS and the mitral valve is most frequently affected. Several series of patients have been published demonstrating valvulopathy in patients with the primary APS (Cervera et al., 1991) as well as in SLE (Khamashta et al., 1990).

4.4.2. Vegetations

Non-bacterial vegetations may be combined with valve thickening and are thought to reflect the same pathological process. Libman–Sacks endocarditis, as these lesions are named, may eventually heal with a fibrous plaque, sometimes with focal calcification and marked scarring, thickening and deformity leading to valve dysfunction. Regurgitation is common while stenosis is rare and the mitral valve is mainly affected followed by the aortic valve. The tricuspid and pulmonary valves are even less frequently affected. Usually, in APS patients, these valve lesions are not of clinical or hemodynamic significance, but with extensive deformity surgical replacement may be necessary. Thromboembolic events constitute the major danger to the patient with Libman–Sacks endocarditis and can damage brain, kidney and other organs.

4.4.3. Pseudo-infective endocarditis

These patients may present with fever, splinter hemorrhages, cardiac murmurs with echocardiographic evidence of valve vegetations, moderate to high levels of aPL, and repeatedly negative blood cultures (Font et al., 1991).

4.5. Intracardiac thrombus

Several patients with aPL have been reported who developed thrombi in the ventricular cavities (O'Neill et al., 1995). Clinically, patients may present with systemic or pulmonary embolic symptoms, depending on the location of the thrombus (right or left ventricle). Thrombus tends to form on akinetic segments of the ventricle.

4.6. Cyanotic congenital heart disease

A few patients with cyanotic congenital heart disease and elevated aCL have been published (Martínez-Lavín et al., 1995).

5. Pulmonary manifestations

5.1. Pulmonary embolism and infarction

Pulmonary embolism and complicating infarctions are frequently seen in APS patients, occurring in approximately one-third of patients presenting with DVT. Rarely, thromboembolic pulmonary hypertension (PHT) supervenes (Asherson et al., 1990).

5.2. Major pulmonary arterial thrombosis

Although cerebral and peripheral vessels are most commonly involved in aPL-induced thrombosis, major pulmonary arterial occlusion is distinctly uncommon (Luchi et al., 1992).

5.3. Pulmonary microthrombosis

Although originally thought to be the cause of PHT in aPL-positive patients, this complication is uncommon. Several patients who demonstrated small vessel occlusive disease of the pulmonary vasculature have been documented, all with histopathological evidence provided (Gertner and Lie, 1993).

5.4. Pulmonary ''capillaritis''

Gertner and Lie (1993) coined this term to describe aPL patients who on histopathological examination also often demonstrated alveolar hemorrhage and microvascular thromboses.

5.5. Pulmonary hypertension

A relationship exists between thromboembolic PHT and aPL for obvious reasons. It is also of interest that a percentage of patients with plexogenic PHT of the ''primary'' variety may also demonstrate aPL in low to moderate titers, leading investigators suspect that either the aPL may be just another immunological accompaniment of the PHT or perhaps be an indication again of diffuse pulmonary endothelial cell damage (Asherson and Cervera, 1995).

6. Renal manifestations

6.1. Renal vein thrombosis

The first two such cases were reported in 1984 (Asherson et al., 1984) and in the same year, Mintz et al. (1984) pointed out that renal vein thrombosis was more frequently encountered in patients with nephrotic syndrome (when a leak of antithrombin III occurs, predisposing to thrombosis), as well as in patients who had suffered a previous DVT of the limbs.

6.2. Major renal artery occlusions

Renal artery trunk lesions have also been documented both in SLE as well as in primary APS patients. Hypertension is a not infrequent accompaniment of this condition and may be severe and on occasion may result in oliguric renal failure (D'Cruz, 2005).

6.3. Renal infarction

Consequent on vascular occlusive disease of renal vessels, renal infarction has been documented by several investigators (Sonpal et al., 1993). Although widely resulting from in situ thrombosis of the renal artery or one of its branches, as a complication of renal artery ''stenosis'' or fibromuscular dysplasia. it may rarely result from a possible embolus originating from a cardiac valve lesion (Mandreoli and Zucchelli, 1993).

6.4. Thrombotic glomerular microangiopathy

In SLE patients it has been clearly demonstrated that the presence of aPL associates strongly with glomerular capillary thrombosis and that this occurs more frequently in patients suffering from proliferative glomerular nephritis. Progression to

glomerular sclerosis also seems to be more frequent in those patients who present initially with capillary thrombosis. Additionally, several reports of glomerular microangiopathy have been documented in patients with primary APS (Amigo et al., 1992), as well as in the catastrophic APS (Bucciarelli et al., 2006).

7. Adrenal manifestations

The first reports of an association between the aPL and the adrenal gland, manifesting as hypoadrenalism (usually acute) appeared in 1989 (Asherson and Hughes, 1989) and, at a special meeting commemorating the 200th anniversary of the birth of Thomas Addison, a comprehensive review of 41 reported cases was given (Asherson, 1994). Histopathologically, the adrenal failure is caused by adrenal hemorrhage and infarction consequent on the hypocoagulable state. Thrombosis of adrenal veins as the primary event occurs and the hemorrhagic infarction ensues as a secondary phenomenon. The adrenal glands may be enlarged on abdominal CT but over the course of time, adrenal atrophy ensues (Espinosa et al., 2003).

8. Hepatic and digestive manifestations

8.1. Hepatic manifestations

Several hepatic manifestations have been described in patients with aPL. These include Budd–Chiari syndrome (Espinosa et al., 2001), portal hypertension, obstruction of small hepatic veins (hepatic veno-occlusive disease), nodular regenerative hyperplasia (Morlà et al., 1999), and hepatic infarctions, among others. Most of these lesions are due to vascular lesions of liver microcirculation.

8.2. Esophageal necrosis

A patient with a primary APS who thrombosed vessels at the lower end of the esophagus, resulting in necrosis, septic mediastinitis, and death, has been documented (Cappell, 1994).

8.3. Gastric ulceration

Progressive gastric ulceration with necrosis in a patient presenting with severe abdominal pain was found to be due to widespread occlusive vascular disease involving veins, small arteries, and arterioles in one patient (Kalman et al., 1996).

8.4. Small and large bowel vascular occlusions

Several cases of large bowel and intestinal infarctions in patients with aPL have been reported. Peritonitis is a not uncommon accompaniment. Severe gastrointestinal hemorrhage may also result from bowel ischemia or from an atypical duodenal ulcer (Cervera et al., 2007).

8.5. Mesenteric inflammatory vaso-occlusive disease

This condition primarily affects veins and venules of the bowel and mesentery resulting in ischemic injury; in almost 50% of cases thus far described, these are primary or idiopathic. Small bowel infarction has been described due to mesenteric inflammatory vaso-occlusive disease in patients with primary APS (Gül et al., 1996).

8.6. Pancreatitis

Abdominal pain in patients with the APS may be due to pancreatic involvement by the microangiopathy characteristic of the aPL. The presentation may be acute, with abdominal pain and vomiting. Pancreatic involvement was noted in several patients with catastrophic APS recently reviewed (Bucciarelli et al., 2006). However, in only very few was the diagnosis made clinically.

8.7. Cholecystitis

A few patients presenting with acute cholecystitis in the absence of gallstones have been reported in the course of catastrophic APS (Bucciarelli et al., 2006).

8.8. Occlusion of splenic vessels

Occlusion of splenic vessels has been reported in combination with other vascular occlusions. Splenic infarction may supervene (Cervera et al., 2007).

9. Dermatologic manifestations

9.1. Cutaneous necrosis

Widespread cutaneous necrosis associated with massive thrombosis of small and medium-sized dermal vessels has been reported in patients with the APS (Asherson and Cervera, 1993a, b).

9.2. Macules and nodules

Erythematous macules and painful skin nodules occurring in aPL-positive patients have been reported. These lesions are due to thrombotic skin disease, and are located on the palms, soles, and fingers and do not disappear on pressure. These painful lesions have been reported as improving with salicylate therapy.

9.3. Multiple subungual hemorrhages

These have been reported in the APS, in the presence of pseudo-infective endocarditis (Font et al., 1991), as well as in the catastrophic APS (Bucciarelli et al., 2006).

9.4. Gangrene and digital necrosis

Cutaneous ischemic symptoms may culminate in digital gangrene and aPL-associated gangrene, particularly in SLE and this must be distinguished from vasculitis, cryoglobulinemia, or disseminated intravascular coagulation. It is one of the hallmarks of the cutaneous complication of the catastrophic APS (Asherson et al., 2007).

9.5. Other

Other dermatological syndromes, such as anetoderma, necrosis of the skin associated with cryofibrinogenemia and diabetes mellitus, and pyoderma gangrenosum, have also been reported in patients with aPL (Asherson and Cervera, 1993a, b).

10. Osteoarticular manifestations

10.1. Avascular necrosis of bone

The etiology of avascular necrosis (AVN) of bone in SLE patients is probably multifactorial and several risk factors have been suggested, including Raynaud's phenomenon, glucocorticoid therapy, particularly in these patients developing features of Cushing's syndrome and vasculitis. A possible link between AVN and aPL was postulated in 1985 (Asherson et al., 1985) and this has been strengthened by reports of AVN in patients with the primary APS who had not been exposed to glucocorticoid therapy at all (Tektonidou et al., 2003).

10.2. Bone marrow necrosis

This is an unusual condition characterized by pancytopenia. Its association with aPL was first documented by Bulvik et al. (1995) and since then several further cases have been reported.

11. Hematologic manifestations

11.1. Thrombocytopenia

Varying degrees of thrombocytopenia occur with the APS in 20–40% of patients. It is usually moderate, ranging from 75 to 130×10^9/liter in most patients and is not associated with

hemorrhagic phenomena. Occasionally, however, it may be severe, and the differential diagnosis then lies between idiopathic thrombocytopenic purpura and APS. Thrombotic events are unusual with very low platelet counts, but have been reported with aPL. Thrombocytopenia was included in the initial criteria used to diagnose APS but was excluded in the Sapporo criteria. However, thrombocytopenia was suggested to be included as a "minor" criteria for APS in the 9th International aPL Symposium held in Tours, France (Wilson et al., 2001) and as an APS-associated manifestation in the 11th International aPL Symposium held in Sydney, Australia (Miyakis et al., 2006).

Thrombocytopenia can be due to platelet aggregation caused by aPL antibodies binding through β_2 glycoprotein I. In addition, a high prevalence of specific antiplatelet antibodies has been found in APS patients even in those without thrombocytopenia (Macci et al., 1997).

11.2. Coombs' positivity and hemolytic anemia

Several studies have noted the frequent findings of a positive direct Coombs' test in patients with aPL (Cervera et al., 1990). Some data suggest that the aCL may be capable of recognizing a phospholipid epitope on the surface of the red blood cell. The association of hemolytic anemia with the IgM isotype of aCL has also been demonstrated (Cervera et al., 1990).

The association of both autoimmune thrombocytopenia and hemolytic anemia (Evans syndrome) can be found in 5–10% of patients with APS (Asherson et al., 1989).

11.3. Thrombotic microangiopathic hemolytic anemia

Thrombotic thrombocytopenic purpura (TTP), hemolytic–uremic syndrome and HELLP syndrome (acronym of hemolysis, elevated liver enzymes, and low platelets) occurring in pregnancy are different presentations of the thrombotic microangiopathic hemolytic anemia that can be found in APS patients. In several cases, TTP has been reported in APS patients associated to anti-ADAMTS-13 antibodies.

11.4. Pure red cell aplasia

A very infrequent association between pure red cell aplasia and APS has been described. However, some of these cases could be actually due to human parvovirus B19 infection.

11.5. Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) has been rarely described in both primary APS and APS associated with SLE, but laboratory features of DIC are more frequently seen in patients with the catastrophic variant of APS.

11.6. Neutropenia

A significant association of IgM aCL with neutropenia has also been described (Cervera et al., 1990). Neutropenia is more frequent in APS associated with SLE than in primary APS. Lymphopenia, however, does not correlate with APS.

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CHAPTER 9

Obstetric Manifestations of the Antiphospholipid Syndrome

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1. Introduction

The antiphospholipid syndrome (APS) was first described in patients with mid-trimester fetal losses (Soulier and Boffa, 1980), supporting the impact of the obstetric manifestation in this condition.

When classification criteria of APS were drawn up in 1999 (Wilson et al., 1999) and revised in 2006 (Miyakis et al., 2006), the obstetric manifestations were described as one out of the two clinical aspects characterizing the syndrome. From a practical point of view, the correct definition of the APS-related obstetric complications and the consequent application of proper management had the greatest impact on the life of these patients, giving back to them, in the majority of cases, the possibility of planning their family life.

Under the definition of "obstetric APS" several pathological manifestations of pregnancy are included: early miscarriages, fetal losses, early severe preeclampsia with placental insufficiency and growth restriction and even the HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, recently classified with the microangiopathic APS (Asherson, 2006). The wide spectrum of APS pregnancy complications suggests that more than one pathogenic mechanism might be involved, perhaps reflecting several possible interactions between antiphospholipid antibodies (aPL) and the uteroplacental unit. In this respect aPL-related thrombosis is one but certainly not the only pathologic phenomenon occurring at placental level. The observation that pregnancy losses tend to recur in about 20% of the patients despite careful management and despite a treatment based on the actual guidelines, suggests that part of aPL-mediated pregnancy damage remains still undefined and therefore not adequately treated.

Another aspect that has to be taken into account is the clinical setting of patients with obstetric APS. The occurrence of one or more fetal losses in a patient with systemic lupus erythematosus (SLE) and deep vein thrombosis (DVT) appears to be a different condition from that in a patient with recurrent early miscarriages or one fetal death in the absence of other medical problems. It seems that these two conditions are only scarcely comparable and that the recurrence rate is probably higher in the first one (Branch, 2004). Despite these observations, what we know about the treatment of such patients is directly derived from clinical trials that mainly include patients with early miscarriages only, and from which patients with autoimmune diseases and/or thrombosis were excluded. Therefore much of the responsibility of the final outcome is still left to careful monitoring of the pregnant patient and, when needed, to a prompt decision of the delivery

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time, causing iatrogenic preterm delivery but increasing the number of live births.

2. Early miscarriages

In an unselected obstetric population, 10–15% of pregnancies end in spontaneous abortion, mainly occurring during the pre-embryonic (<6 menstrual weeks of gestation) or embryonic period (from 6 to 9 menstrual weeks of gestation), while the rate of fetal loss, after 14 weeks of gestation, is very low. In addition, the recurrence of early miscarriages in the general population is not unusual, being recorded in 1/100 or 1/200 women. In this population, which is usually without significant medical history, variable titers of positive aPL have been found in about 20% of cases.

However, the attribution of early recurrent miscarriages to aPL-mediated damage implies the exclusion of several other possible causes. Chromosomal abnormalities, which often impair embryo formation, account for more than half of sporadic (pre)embryonic losses. In contrast, genetic abnormalities of the conceptus are less common in women with three or more consecutive miscarriages. The need for at least three losses in the first 9 weeks of gestation as a criterion to enter a formal clinical diagnosis of APS is probably based on this observation; this excludes most of the losses due to genetic abnormalities that can confuse the clinical picture. Other possible confounding factors are uterine anomalies, luteal phase insufficiency, cervical infections, and thyroid hormone dysfunctions. All these possible conditions should be carefully considered in the diagnostic workup of patients with recurrent early miscarriages and positive aPL.

The original clinical observations leading to the APS definition were based on patients with mid-trimester losses; this is unsurprising since such losses have a great impact on patients and for this reason they draw the attention of physicians. Nevertheless, recurrent early miscarriages, often misdiagnosed because they are frequent events in the general obstetric population, can be considered to be the most frequently occurring pregnancy losses associated with aPL. In fact clinical trials on obstetric APS, which still provide the only evidence-based information on treatment, include almost only patients with early pregnancy loss. This is justified by the fact that: (1) these studies were conducted by obstetric teams, who mainly see patients in this clinical setting, and (2) it is always easier to include uncomplicated patients in randomized clinical trials rather than those with several medical aspects to be monitored.

Despite the relative clinical homogeneity of the recruited population, the results of these studies are in some ways discordant. For example, the effectiveness of heparin is only shown by two clinical trials (Kutteh, 1996; Rai et al., 1997), while in others aspirin alone or even clinical monitoring alone seem to achieve the same success rate (Pattison et al., 2000; Farquharson et al., 2002).

It is not easy to interpreter these discrepancies; a possible explanation could be difficulties in the laboratory definition of patients. In fact, the pathogenic potentials of different antibodies in term of specificity (anticardiolipin vs. anti-β_2 glycoprotein I (anti-β_2GPI) antibodies) and in term of isotypes (IgG vs. IgM/IgA) might be of some importance. In addition, the well-known lack of standardization of aPL immunoassays and of lupus anticoagulant might also have had some effect on the recruitment of patients with different biological features in the above-mentioned trials conducted between 1996 and 2002. It is hoped that the advent of second- and third-generation assays and the availability of new reference materials (Meroni et al., 2004b) will overcome this problem, helping to focus on the population with real aPL-related problems among the wide group of women with recurrent miscarriages.

3. Fetal death

Historically, repeated late fetal deaths were the first reported obstetrical complication associated with aPL; they were sometimes observed in patients with thrombotic episodes (Soulier and Boffa, 1980; Carreras et al., 1981). In fact, in everyday clinical practice, late pregnancy failures are a frequent

problem for women with aPL and represent one of the clinical classification criteria for APS.

Among the formal classification criteria, fetal death is considered the most specific symptom defining obstetric APS, while recurrent early abortions may be the most sensitive (Wilson et al., 1999). In patients suffering recurrent pregnancy loss, the specificity of fetal death for the presence of aPL was shown to be up to 76% compared with only 6% for two or more pre-embryonic pregnancy losses (Oshiro et al., 1996). Today, according to what is known about the biological development of the embryo, the fetal period is defined as starting at the 10^{th} week of gestation and lasting until delivery.

In the general population about 2% of pregnancy losses seem to occur between 14 and 20 weeks of gestation, which therefore appears to be the most critical period of fetal life (Goldstein, 1994). Including both fetal and neonatal periods, it is estimated that up to 5% of normal pregnancies end in loss (Frias et al., 2004).

Fetal damage may be reasonably due to the presence of aPL only after exclusion of other possible causes. In this respect heritable thrombophilias such as factor V or factor II Leiden mutation, antithrombin, protein C or protein S deficiency, have been associated with fetal losses, although their role in the fetal damage is still debated (Rasmussen and Ravn, 2004; Dizon-Townson et al., 2005).

According to the revised classification criteria (Miyakis et al., 2006), patients with multiple positivity for aPL (lupus anticoagulant, anticardiolipin antibodies, and anti-β_2GPI antibodies) are at higher risk of thrombosis occurrence and recurrence. The same patient profile is at higher risk of fetal death with uteroplacental thrombosis, infarction, and vasculopathy. This event may occur even when patients are adequately treated and in fact represents the majority of pregnancy loss recurrence recorded despite treatment with heparin and low-dose aspirin (Vianna et al., 1994).

Thrombosis occurrence is probably not the only pathogenic mechanism responsible for fetal losses, since several groups have shown a direct binding of aPL to trophoblastic surface via β_2GPI together with a local inflammatory process including complement activation (Meroni et al., 2004a; Stone et al., 2006). In vivo and in vitro models of aPL-mediated defective placentation possibly causing fetal death are detailed in a different chapter of this book.

4. Preeclampsia and HELLP syndrome

Preeclampsia is a multisystem disorder of unknown cause that is unique to human pregnancy. It is characterized by abnormal vascular response to placentation that is associated with: increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, endothelial cell dysfunction.

Preeclampsia is generally defined as increased blood pressure associated with proteinuria in pregnancy. Diagnostic blood pressures include either a systolic blood pressure greater than or equal to 140 mmHg or a diastolic blood pressure greater than or equal to 90 mmHg on at least two occasions (at least 4 hours apart). Proteinuria is defined as the excretion of 300 mg of protein or greater in a 24-hour specimen (ACOG practice bulletin, 2002).

Preeclampsia can be divided into two groups: mild and severe. It is classified as severe when one or more of the following criteria are present: (1) systolic blood pressure of 160 mmHg or greater or diastolic blood pressure of 110 mmHg or greater; (2) proteinuria of 5 g or more in 24 hours; (3) elevated serum creatinine (>1.2 mg/dL); (4) elevated liver enzymes; (5) persistent headache or visual disturbance; (6) persistent epigastric or right upper quadrant pain; (7) platelet count less than 100 000/mm^3 and/or evidence of microangiopathic hemolytic anemia; (8) preeclampsia.

HELLP syndrome can be considered a variant of preeclampsia. HELLP is an acronym for hemolysis, elevated liver enzymes, low platelets; these findings often occur together, so identifying the classic HELLP syndrome, but often only one or some of these findings accompanied preeclampsia.

Preeclampsia is a major obstetric problem leading to substantial maternal and perinatal morbidity and mortality worldwide, especially in

developing countries. Maternal and perinatal outcomes in preeclampsia depend on one or more of the following: gestational age at time of disease onset, the severity of disease, the quality of management, and the presence or absence of pre-existing medical disorders. Maternal and perinatal outcomes are usually favorable in women with mild preeclampsia beyond 36 weeks' gestation. By contrast, maternal and perinatal morbidities and mortalities are increased in women who develop the disorder before 33 weeks' gestation and in those with pre-existing medical disorders.

The relationship between aPL and preeclampsia is debatable. Branch and associates in 1989 first reported an association between severe preeclampsia at <34 weeks' gestation and aPL (Branch et al., 1989). This report included patients with severe preeclampsia and those with SLE and a history of thromboembolism. In several studies the occurrence in patients with primary or secondary APS is high and in obstetric populations with preeclampsia a high frequency of aPL-positive patients has been observed (Branch et al., 1985, 1992; Lockshin et al., 1985; Caruso et al., 1993; Lima et al., 1996). In addition, the presence of anti-β_2GPI was shown to be predictive for severe early preeclampsia and eclampsia in a general obstetric population (Faden et al., 1997). Other studies have found no relationship between preeclampsia and aPL in either a general population of women with preeclampsia or in women at risk for the development of preeclampsia (Scott, 1987; Harris and Spinnato, 1991; Out et al., 1992; Lynch et al., 1994; Uncu et al., 1996; D'Anna et al., 1997; Martinez-Abundis et al., 1999; Dreyfus et al., 2001; Matthiesen et al., 2001; Lee et al., 2003). Besides the gestational time of preeclampsia, routine term (after 37 weeks' gestation) preeclampsia has not been associated with increased levels of aPL.

These discrepancies in the results among studies may be related to a lack of standardization of the aPL assays and different thresholds for the definition of a positive test for aPL. Moreover, low positive anti-cardiolipin (aCL) levels are of questionable clinical significance (Dekker et al., 1995; Van Pampus et al., 1999).

On the other hand, there is a substantial risk for developing preeclampsia in women meeting clinical and laboratory criteria for APS, and the explanation lies in the relative infrequency of APS compared with the relative frequency of preeclampsia.

The median rate of preeclampsia in women with APS (including women with SLE and prior thrombosis) is 20–50% despite the treatment; half of these patients had severe preeclampsia and this may explain the high rate of preterm delivery in APS patients (Branch et al., 1985, 1992; Lockshin et al., 1985; Caruso et al., 1993; Lima et al., 1996).

It is worth noting that the clinical criteria "one or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (a) eclampsia or severe preeclampsia defined according to standard definition or (b) recognised features of placental insufficiency" is included in the classical definition of APS syndrome.

Preeclampsia is a pregnancy complication related to uteroplacental insufficiency. There is evidence supporting a causal or pathogenetic model of superficial placentation determined by immune maladaptation, with subsequently reduced concentrations of angiogenic growth factors and increased placental debris in the maternal circulation resulting in a (mainly hypertensive) maternal inflammatory response.

The final phenotype, maternal pre-eclamptic syndrome, is further modulated by pre-existing maternal cardiovascular or metabolic fitness. Cytotrophoblasts in spiral arteries, apoptosis, or increased syncytiotrophoblast apoptosis could determine fibrin deposition, as well as platelet activation (Salafia et al., 1998; Ishihara et al., 2002). In addition, annexin A5 production by trophoblasts is reduced in preeclampsia, possibly as a result of inflammatory cytokine and free-radical activity. The degree of annexin A5 reduction correlates with the increase in markers of coagulation activation, maternal disease severity, and severity of intrauterine growth restriction. Antiphospholipid antibodies have been shown to inhibit differentiation, decrease proliferation, migration, and gonadotropin synthesis (GnRH-induced human chorionic gonadotropin) (Di Simone et al., 2000), increase apoptosis, and retard invasion of the endovascular trophoblast that forms plugs in the

maternal spiral arteries (Di Simone et al., 2000; Sebire et al., 2002; Van Horn et al., 2004). Displacement of protective annexin A5 protein allows deposition of fibrin on the trophoblast cell surface and fixation of complement, leading to generation of C5a, increased inflammation, and elevated levels of tumor necrosis factor (Holers et al., 2002). Shamonki and colleagues demonstrated that excessive complement activation (causing increased deposition of complement factors C4d and C3b) is associated with placental injury in patients with aPL. They also reported a correlation between placental hystopathologic features (which include villous infarction, decidual vasculopathy, decidual vascular thrombosis) and complement deposition (C4d) in the trophoblastic cytoplasm, cell membrane, and basement membrane. (Shamonki et al., 2007) (Fig. 1).

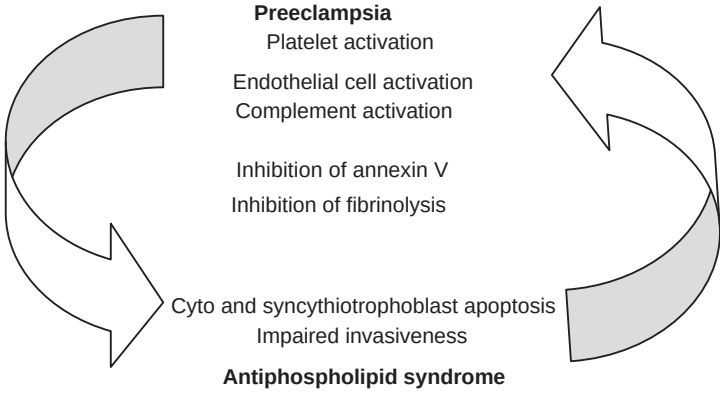

Figure 1. Pathogenesis of preeclampsia and antiphospholipid syndrome.

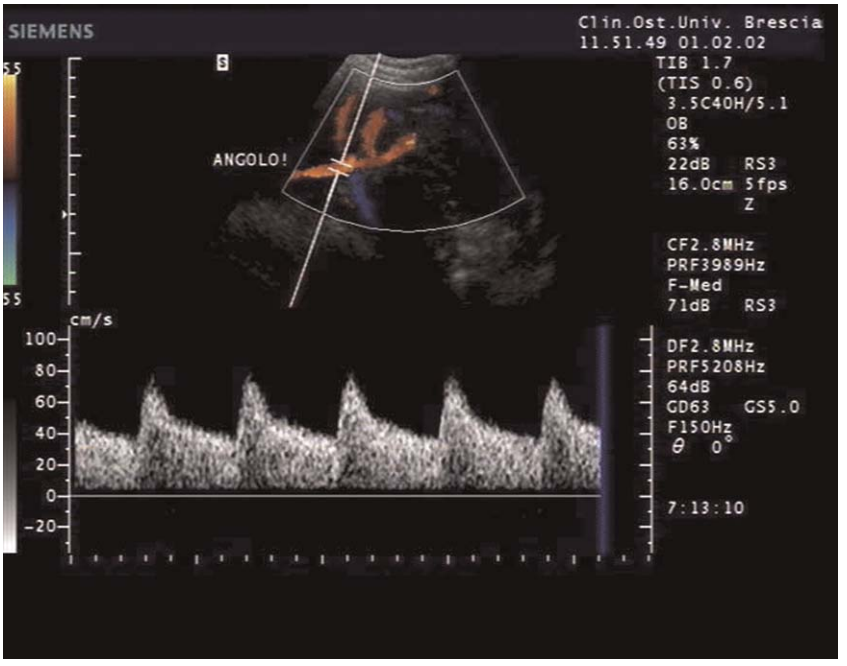

Figure 2. Normal flow in uterine artery. (See Colour Plate Section.)

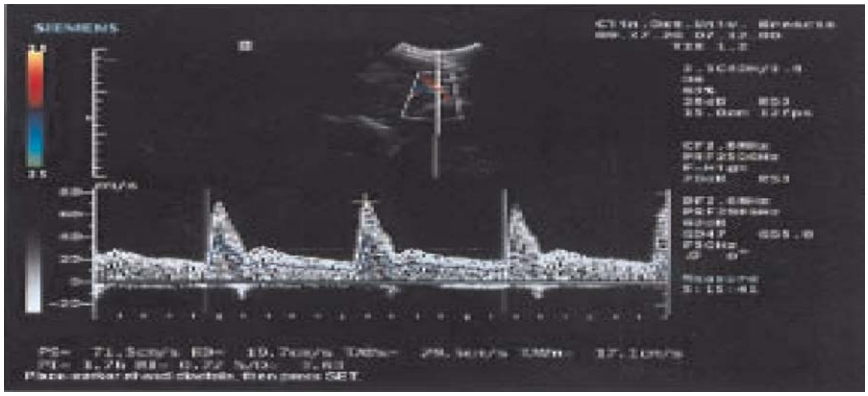

Figure 3. Pathological flow in uterine artery. (See Colour Plate Section.)

Poor placentation has been demonstrated to impair the physiological change of spiral arteries into low-resistance vessels during the first half of pregnancy. The absence of this remodeling involves the persistence of high-pressure blood flow, impairing uteroplacental blood flow. To study uteroplacental flow the best non-invasive indirect method is uterine artery Doppler velocimetry. The persistence of high resistance in uterine arteries at 24 weeks of pregnancy may reflect the failure of uterine vascular adaptation and would select a subgroup of patients at higher risk of pregnancy complications such as preeclampsia, intrauterine fetal death, and/or fetal intrauterine growth retardation (Venkat-Raman et al., 2001).

The increase in flow resistance results in an abnormal waveform pattern, comprising an increased bilateral resistance index or the persistence of the uni–bilateral protodiastolic notch (Figs. 2 and 3). Several studies have been performed to detect the predictive role of uterine artery Doppler velocimetry and pregnancy outcome in APS patients: all of them underlined the need for intensified surveillance and monitoring of pregnancy in the case of Doppler abnormalities (Blumenfeld et al., 1991; Benifla et al., 1992; Caruso et al., 1993; Meizner et al., 1988; Bar et al., 2001; Farrel and Dawson, 2001; Venkat-Raman et al., 2001; Bats et al., 2004; Le Thi Huong et al., 2006; De Carolis et al., 2007). Moreover, the normal uterine artery resistence index (RI) had good negative predictive value and could give early prediction of a good pregnancy outcome, allowing a reduction of antenatal care in terms of visits and obstetric surveillance and giving these APS pregnant patients reassuring counseling.

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CHAPTER 10

Pediatric Antiphospholipid Syndrome

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1. Introduction

The antiphospholipid syndrome (APS) is a multisystem autoimmune disease characterized by vascular thrombosis, recurrent fetal loss, thrombocytopenia, and other clinical manifestations in the presence of persistent circulating antiphospholipid antibodies (aPL) (Levine et al., 2002). APS in children may present in the neonatal period due to the transplacental passage of maternal aPL or later during childhood and adolescence due to de novo synthesis of aPL. Most of the clinical features which can occur in adults with aPL have also been described in pediatric population, however, clinical expression of aPL in children is modified by several characteristics of the pediatric period such as immaturity of the immune and other organ systems (e.g. developing hemostasis and central nervous system, establishing oral tolerance), absent common prothrombotic risk factors present in adults (such as atherosclerosis, cigarette smoking, hypertension, and use of oral contraceptives), no pregnancy morbidity, routine immunizations, and increased exposure to viral and bacterial infections (Avčin and Silverman, 2007; Ravelli and Martini, 2007).

The clinically most useful aPL for identifying patients with APS are anticardiolipin antibodies (aCL), anti-β_2 glycoprotein I antibodies (anti-β_2GPI), and lupus anticoagulant (LAC). Antiphospholipid antibodies possess different pathogenic properties such as effects on the coagulation pathway, endothelium and platelets as well as possible effects on neural tissues and complement pathways (Mackworth-Young, 2004; Vega-Ostertag and Pierangeli, 2007). The pathogenic mechanisms of pediatric APS have not been thoroughly investigated, but it appears to be the same as in adults. However, there is some evidence for alternative immune responses of the developing immune system to the particular antigenic challenges, which can result in specific aPL production during childhood (Ambrožič et al., 2002).

2. Epidemiology

The actual prevalence of APS in the pediatric population is difficult to estimate since there are no validated criteria and diagnosis rests on extension of adult guidelines and clinical judgment. Current consensus criteria for the classification of APS in the adult population designate patients who have vascular thrombosis or recurrent fetal losses, accompanied by elevated titers of aCL, anti-β_2GPI or LAC (Miyakis et al., 2006). APS in children has been largely reported in patients with thromboses and less frequently in association with isolated neurological or hematological manifestations (Gattorno et al., 2003; Berkun et al., 2006).

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Recurrent fetal losses, which represent one of the most important clinical criteria for APS in adults, are not applicable for pediatric population and it is possible that current consensus criteria may fail to recognize a subgroup of pediatric patients who do not meet consensus clinical criteria for APS, but demonstrate typical non-thrombotic clinical manifestations and fulfill the laboratory criteria for APS (Avčin et al., 2002a). Pediatric APS is classified as primary, isolated form and associated with underlying disease, most commonly systemic autoimmune disease and rarely malignancy or other underlying disorder. This distinction is not very stringent, however, since children that presented with primary APS may develop overt systemic lupus erythematosus (SLE) during follow-up (Gattorno et al., 2003).

2.1. Primary antiphospholipid syndrome

The prevalence of primary APS in the pediatric population is not known, but it appears to be less common than in adults. Among the 121 children with aPL-related thrombosis included in an international registry of pediatric patients with APS (Ped-APS Registry; a collaborative project of the European Forum on aPL and Lupus Working Group of Paediatric Rheumatology European Society), children with primary APS represented 49.5% of all cases (Avčin et al., 2008b), which is slightly lower than in adults with primary APS reported as 53–57% of all APS patients (Cervera et al., 2002; Garcia-Carrasco et al., 2007).

Although the incidence of thrombosis in children is significantly less than that in adults, the proportion of aPL-related thrombosis in children appears to be higher than that in the adult population. Manco-Johnson and Nuss (1995) identified LAC in 25% of 78 consecutive children who were diagnosed with thromboses in their institution. In addition, Tavil et al. (2007) reported positive aPL in 11.6% of 138 children with thrombosis and aPL were identified as the second most common prothrombotic risk factor after factor V Leiden mutation among children with thrombosis.

2.2. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is the autoimmune disease in which aPL occurs most frequently. A meta-analysis of the published studies that investigated the prevalence and clinical significance of aPL in pediatric SLE showed a global prevalence of 44% for aCL, 40% for anti-β_2GPI, and 22% for LAC (Avčin and Silverman, 2007). The reported frequencies of aCL and LAC in pediatric SLE patients have ranged from 19% to 87% and from 10% to 62%, respectively (Shergy et al., 1988; Ravelli et al., 1994; Gattorno et al., 1995; Seaman et al., 1995; Berube et al., 1998; Avčin et al., 2008a). This wide variability in the frequency of aPL reported in pediatric SLE may be due to differing sensitivities of assays and heterogeneity in the patient population regarding the disease duration, clinical features, and disease activity.

A variety of clinical features have been reported to be present in aPL-positive children with SLE and some pediatric studies suggested significant association of persistently positive aPL with SLE disease activity and long-term damage (Brunner et al., 2002; Avčin et al., 2008a; Descloux et al., 2008). A large Canadian study of 137 children with SLE found that SLE disease activity index (SLEDAI) score correlated with aCL and anti-β_2GPI levels, but no association with irreversible disease damage was found (Avčin et al., 2008a). In contrast, Descloux et al. (2008) observed in a retrospective study of 58 children with SLE that the risk of disease damage assessed by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage index (SDI) in aPL-positive patients was three times higher than in aPL-negative patients. These findings suggest that aPL can modify the clinical expression and long-term outcome of pediatric SLE. In a child with SLE, it is recommended that aPL testing be performed at the time of diagnosis and then at least once yearly as part of routine screening.

2.3. Juvenile idiopathic arthritis

The presence of aCL in children with juvenile idiopathic arthritis (JIA) has been investigated in

several cross-sectional studies, which reported a frequency ranging from 7% to 53% (Caporali et al., 1991; Serra et al., 1999). A meta-analysis of the published studies of aCL in children with JIA yielded a global prevalence of 30% (Avčin et al., 2002a). A prospective study of 28 children with JIA demonstrated persistently positive aCL in 21%, anti-β_2GPI in 4% and no patient with persistently positive LAC (Avčin et al., 2002b). Most studies in JIA found no association between the presence of aPL and disease activity and no clinical manifestations of APS were observed. There were only two reports of aPL-associated thrombosis in children with JIA (Caporali et al., 1992; Andrews and Hickling, 1997). The limited prothrombotic potential of aPL observed in JIA patients could be partially explained by the low frequency of anti-β_2GPI and LAC, which are reportedly more specific for thrombosis than aCL. Von Landenberg et al. (2003) reported a high incidence of persistent parvovirus B19 infection in aPL-positive children with JIA with no clear clinical significance.

2.4. Infections

Many viral and bacterial infections in childhood may induce production of aPL, which tend to be transient and are generally not associated with clinical manifestations of APS (Shoenfeld et al., 2006; Avčin and Toplak, 2007). The most common infections associated with APS include parvovirus B19, cytomegalovirus, varicella-zoster virus, HIV, streptococcal and staphylococcal infections, gram-negative bacteria, and *Mycoplasma pneumoniae* (Avčin and Toplak, 2007). Because most children suffer from frequent viral and bacterial infections, a high percentage of incidental aPL positivity might be expected for the pediatric population. Since post-infectious aPL tend to be transient, all positive aPL values should be verified on at least two occasions at least 12 weeks apart, preferably at a time when the child has not had a recent infection.

The risk of APS manifestations with post-infectious aPL is not completely absent, however, and there is a growing list of infectious agents known to be ''triggering'' factors for APS. There have been a few pediatric case reports of thromboembolic events associated with *Mycoplasma pneumoniae* infection, which is a common cause of community-acquired pneumonia in children (Witmer et al., 2007; Brown et al., 2008). Another common infectious cause in children, varicella-zoster virus, was described in pediatric cases with cerebrovascular disease (Losurdo et al., 2006) and in association with a distinct clinical entity, purpura fulminans, after chickenpox infection linked to the presence of LAC and acquired protein S deficiency (Josephson et al., 2001; Campanelli et al., 2004).

2.5. Other diseases

The presence of circulating aPL has been demonstrated in a variety of other pediatric autoimmune and non-autoimmune diseases, including juvenile dermatomyositis, Henoch–Schönlein purpura, Kawasaki syndrome, Wegener's granulomatosis, rheumatic fever, insulin-dependent diabetes mellitus, atopic dermatitis, and malignancies (Avčin et al., 2002a; Ravelli and Martini, 2007). In most of these conditions aPL-related clinical manifestations are unusual, and the significance of aPL needs further confirmation.

2.6. Healthy children

Antiphospholipid antibodies can be found in a high percentage of children without any underlying disorder. Such naturally occurring aPL are usually present at low levels and could be the result of previous infections or vaccinations that are common in the pediatric population. The estimated frequency of aCL in healthy children ranges from 3 to 28%, which is higher than in the normal adult population (Rapizzi et al., 2000; Avčin et al., 2001; Cabiedes et al., 2002). The frequency of anti-β_2GPI in healthy children ranges from 3% to 7% and high levels of anti-β_2GPI seems to be relatively more frequent in preschool children than in adolescents and healthy adults (Avčin et al., 2001; Cabiedes et al., 2002). The risk of future

thrombosis is low in otherwise healthy children who were incidentally found to have positive aPL, but it is prudent to perform a follow-up determination after a time interval of at least 12 weeks. LAC have also been described in apparently healthy children and are usually found incidentally in preoperative coagulation screening as prolonged activated partial thromboplastin time (aPTT). Male et al. (1999) reported that none of 80 asymptomatic children with incidentally found positive LAC experienced clinically relevant complications during a mean follow-up of 2.9 years and over half had normalization of aPTT values.

Age-dependent differences in immune responses are also important considerations when evaluating the results of aPL testing in apparently healthy children. For example, there is growing evidence of postnatal synthesis of anti-β_2GPI in infants, which was originally observed in healthy preschool children and infants with atopic dermatitis (Avčin et al., 2001; Ambrožič et al., 2002). Motta et al. (2006) demonstrated that at 12 months of age, all infants born to aPL-positive mothers were negative for aCL, but 64% (14/22) of infants demonstrated positive anti-β_2GPI, suggesting de novo production. Current evidence suggests that anti-β_2GPI detected in infants have low thrombosis risk and have epitope specificity different from the anti-β_2GPI found in patients with APS (Ambrožič et al., 2002). The production of anti-β_2GPI in infants may be associated with the exaggerated immune response to nutritional antigens or infections and not with an autoimmune disease.

3. Clinical manifestations

Children may present with various aPL-related manifestations that were described also in adults with APS (Avčin et al., 2002a; Ravelli and Martini, 2007). There are some clear differences in the frequency of specific clinical events, since children generally do not have common prothrombotic risk factors present in adults. In particular, various isolated neurological and hematological manifestations may occur more frequently in the pediatric population, while recurrent fetal losses are obviously not a pediatric problem and were reported only in a few exceptional adolescent cases.

3.1. Thromboses

Classical clinical features of APS in pediatric populations include venous, arterial, and small vessel thromboses. Vascular occlusion in APS may involve arteries and veins at any level of the vascular tree and in all organ systems with various clinical presentations. The most frequently reported sites of venous and arterial thrombosis associated with aPL in children are presented in Tables 1 and 2. Recent analysis of the 121 children

Table 1
Venous thrombosis manifestations associated with aPL in children

Vessel involved	Clinical manifestations
Limbs	Deep vein thrombosis
Skin	Livedo reticularis, chronic leg ulcers, superficial thrombophlebitis
Large veins	Superior or inferior vena cava thrombosis
Lungs	Pulmonary thromboembolism, pulmonary hypertension
Brain	Cerebral venous sinus thrombosis
Eyes	Retinal vein thrombosis
Liver	Budd–Chiari syndrome, enzyme elevations
Adrenal glands	Hypoadrenalism, Addison's disease

Table 2
Arterial thrombosis manifestations associated with aPL in children

Vessel involved	Clinical manifestations
Limbs	Ischemia, gangrene
Brain	Stroke, transient ischemic attack, acute ischemic encephalopathy
Eyes	Retinal artery thrombosis
Kidney	Renal artery thrombosis, renal thrombotic microangiopathy
Heart	Myocardial infarction
Liver	Hepatic infarction
Gut	Mesenteric artery thrombosis
Bone	Infarction

with aPL-related thrombosis included in the Ped-APS Registry revealed that 60% of pediatric APS patients presented with venous thrombosis, 32% with arterial thrombosis, 6% with small vessel thrombosis, and 2% with mixed arterial and venous thrombosis (Avčin et al., 2008b). Deep vein thrombosis in the lower extremities represented 40% of all cases included in the Ped-APS Registry, followed by cerebral sinus vein thrombosis in 7%, and portal vein thrombosis in 3%. The most frequent arterial thrombotic event was ischemic stroke in 26% of all cases, followed by peripheral arterial thrombosis in 2% and retinal artery thrombosis in 2%. In total, cerebrovascular disease including sinus vein thrombosis and ischemic stroke was present in 33% of patients enrolled in the Ped-APS Registry, which is significantly higher than reported in adults (16–21%) (Cervera et al., 2002; Garcia-Carrasco et al., 2007). Digital ischemia and renal thrombotic microangiopathy represented the most common small vessel thromboses among pediatric APS patients. Comparisons between different subgroups revealed that patients with primary APS were younger and had higher frequency of arterial thrombotic events, while patients with APS associated with underlying autoimmune disease were older and had higher frequency of venous thrombotic events (Avčin et al., 2008b).

In the entire cohort of children included in the Ped-APS registry, the presence of aCL was detected in 81%, anti-β_2GPI in 67%, and LAC in 72% of patients (Avčin et al., 2008b). Thirty-three per cent of children included in this cohort were positive for all three aPL subtypes and 67% tested negative for one or more of the aPL subtypes, emphasizing the importance of routine testing for multiple aPL subtypes in clinical practice. In a study of 58 children with SLE, Male et al. (2005) reported that positivity for multiple aPL subtypes showed stronger associations with thrombosis than for individual aPL subtypes because of improved specificity. LAC were found the strongest predictor of the risk of thrombosis, while other aPL subtypes provided no additional diagnostic value (Male et al., 2005).

Children with aPL-related thrombosis frequently exhibit concomitant presence of prothrombotic gene mutations supporting the multifactorial pathogenesis of pediatric APS. Berkun et al. (2006) found inherited thrombophilic defects in 45% of 24 children with APS. In a single-center study, Tavil et al. (2007) reported that 69% of children with APS had more than one prothrombotic risk factor other than circulating aPL. Among children included in the Ped-APS Registry, the presence of one or more inherited thrombophilic risk factors was found in 45% of tested patients, most commonly methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, Factor V Leiden mutation, protein S/protein C deficiency, and prothrombin G20210A heterozygosity (Avčin et al., 2008b). Testing for inherited prothrombotic disorders may be particularly important for early recognition of pediatric APS patients with highest risk for recurrent thrombosis that may benefit with intensified and prolonged anticoagulation therapy.

3.2. Non-thrombotic manifestations

Besides vascular occlusion, aPL have been associated with a great variety of non-thrombotic clinical manifestations such as thrombocytopenia, hemolytic anemia, livedo reticularis, chorea, transverse myelitis, epilepsy, heart valve disease, and other rare manifestations (Cervera et al., 2002). Several of these manifestations are frequently found in association with persistent aPL in children, but they are not specific in patients with APS (Avčin et al., 2002a; Ravelli and Martini, 2007). Non-thrombotic manifestations may appear as an isolated clinical entity or along with thrombosis. All children included in the Ped-APS Registry presented with thrombosis and during the disease course 38% of patients developed concomitant hematological manifestations, 18% skin manifestations, and 16% non-thrombotic neurological manifestations. Compared with the adult population, pediatric APS patients exhibited higher frequencies of Evans' syndrome, Raynaud's phenomenon, migraine headache, and chorea (Avčin et al., 2008b).

The most common hematological manifestations associated with aPL in children are Evans' syndrome, thrombocytopenia, autoimmune hemolytic anemia, and leukopenia. Thrombocytopenia is usually mild with platelet counts greater than 50×10^9/L and clinically benign. El-Bostany et al. (2008) evaluated 42 children with immune thrombocytopenic purpura and found elevated concentrations of IgG aCL in 78% and anti-β_2GPI in all chronic cases, while increased serum levels of IgG aCL were observed only in 27% and anti-β_2GPI in 13% of children with acute immune thrombocytopenic purpura. There are only a few reports of children with severe aPL-related thrombocytopenia causing major bleeding. Some studies have reported an increased risk of children with aPL who present with isolated hematological manifestations for the development of future thrombosis or progression to overt SLE and closer follow-up has been suggested for these children (Diz-Küçükkaya et al., 2001; Gattorno et al., 2003; El-Bostany et al., 2008).

Dermatological manifestations of aPL have not been extensively investigated in childhood, but in clinical practice many aPL-positive children present with chronically cold hands and livedo reticularis. Antiphospholipid antibodies were found in 21% of children with primary or secondary Raynaud's phenomenon (Nigrovic et al., 2003). Several other dermatological manifestations have been associated with APS, including skin ulcers due to multiple small vessel occlusions (Fig. 1), cutaneous necrosis, digital gangrene, superficial thrombophlebitis and nailfold infarcts (Cervera et al., 2002; Tomizawa et al., 2003). The most common skin disorders in children included in the Ped-APS Registry were livedo reticularis (6%), Raynaud's phenomenon (6%), and skin ulcers (3%) (Avčin et al., 2008b).

Typical neurological manifestations of APS are ischemic stroke and cerebral sinus vein thrombosis, both caused by thrombotic occlusion of cerebral vessels. Several other neurological manifestations have been linked to aPL, which are not fully explained by the procoagulant effect of aPL and may result from direct interaction between aPL and neuronal tissue or from immune complex deposition in cerebral blood vessel walls (Caronti et al., 1998; Chapman et al., 1999; Steens et al., 2006). Neurological manifestations associated with immune-mediated mechanisms include chorea, seizures, transverse myelopathy, migraine, cerebral ataxia, transient global amnesia, psychosis, and peripheral neuropathy (Angelini et al., 1996). The most common non-thrombotic neurological manifestations in children included in the Ped-APS Registry were migraine headache (7%), chorea (4%), and seizures (3%) (Avčin et al., 2008b). Chorea has been described as an isolated clinical manifestation in children with aPL or in association with SLE (Angelini et al., 1996; Kiechl-Kohlendorfer et al., 1999; Watanabe and Onda, 2004). Recently, a large retrospective cohort study of 137 children with SLE demonstrated an association between LAC and chorea over the disease course, but not between aCL, anti-β_2GPI or LAC and other neuropsychiatric manifestations (Avčin et al., 2008a). The association between aPL and chorea in children has also been supported by several case reports (Gidwani et al., 2007; Wu et al., 2007). The association between childhood seizure disorder and aPL has been reported in two prospective studies, but cerebrovascular disease should be excluded as a cause (Eriksson et al., 2001; Cimaz et al., 2002). There has been controversy concerning a possible association of aPL and migraine, which has not been confirmed in a prospective study in an unselected group of children with migraine (Avčin et al., 2004).

Antiphospholipid antibodies have also been implicated in the pathogenesis of some orthopedic disorders such as avascular necrosis of bone, nontraumatic fractures, and bone marrow necrosis (Vasoo et al., 2005). Skeletal involvement might be an underrecognized manifestation of APS which presumably develops as a consequence of the microvascular thrombosis leading to bone microinfarcts, osteonecrosis, as well as fractures. Association between aPL and Perthes' disease has been suggested in the pediatric population (Gorshtein and Levy, 2007).

Echocardiographic studies have disclosed heart valve abnormalities resembling Libman–Sacks endocarditis in 11% of adult patients with APS (Cervera et al., 2002), but the frequency of this complication in pediatric APS is unknown.

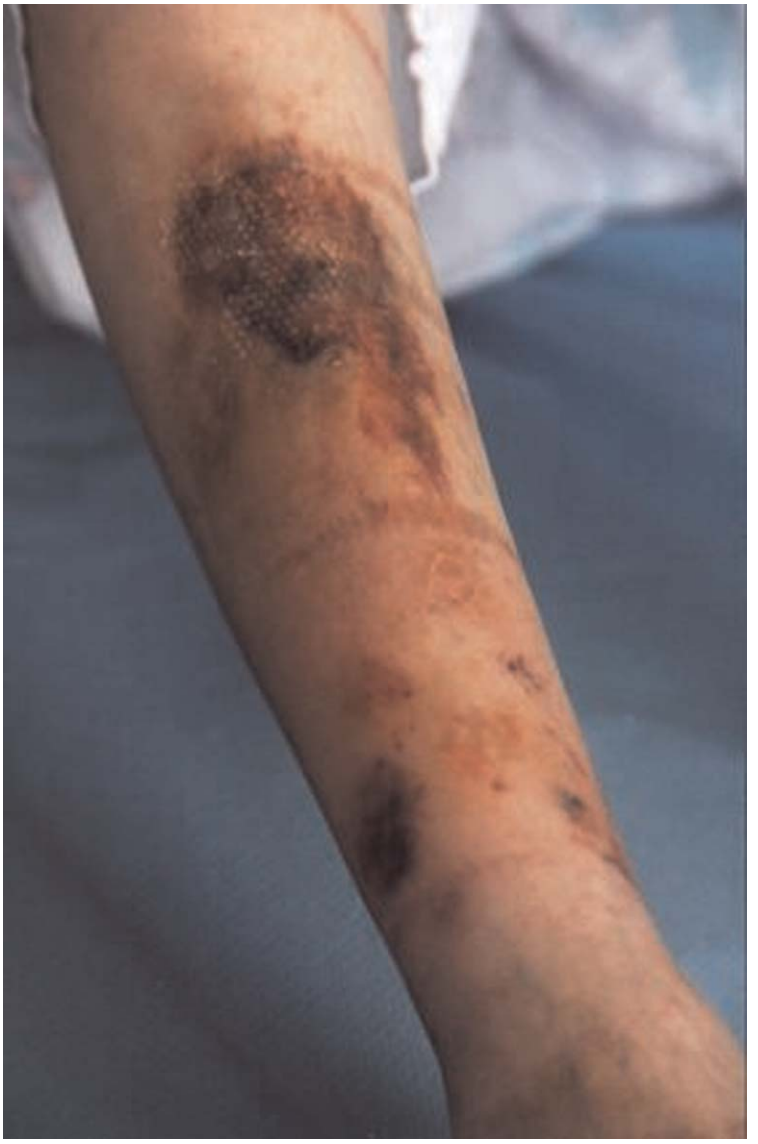

Figure 1. Chronic leg ulcers in a child with systemic lupus erythematosus and positive antiphospholipid antibodies. (See Colour Plate Section.)

3.3. Catastrophic antiphospholipid syndrome

Catastrophic APS is a rare, potential life-threatening variant of APS characterized by aggressive microvascular occlusive disease involving multiple organs. This syndrome is defined as clinical involvement of at least three organ systems over a very short period of time (less than a week) with histopathological evidence of small vessel occlusion in at least one organ system and laboratory confirmation of the presence of aPL (Asherson et al., 2003; Cervera et al., 2005). Thrombosis of large vessels is less common in catastrophic APS, but may occur together with small vessel occlusion. Most commonly affected organ systems include kidney, lung, central nervous system, heart, and skin. Catastrophic APS represents less

than 1% of adult patients with APS and has also been occasionally reported in pediatric patients, mostly associated with preceding infections, surgery, or lupus flare as a precipitating event (Falcini et al., 1997; Olivier et al., 2006; Park et al., 2007).

A separate subset of APS termed ''microangiopathic antiphospholipid-associated syndrome'' was recently proposed to emphasize different conditions affecting the microvasculature that might also demonstrate aPL positivity, such as thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, and related syndromes (Asherson et al., 2007). The pathogenic role of aPL in these clinical conditions remains controversial and patients may exist in whom the presence of aPL positivity just reflect the induction of aPL as an immune response to the endothelial damage or preceding infection. Recently, microangiopathic antiphospholipid-associated syndrome with rapidly progressive aPL-associated thrombotic microangiopathy was reported in a child with atypical hemolytic uremic syndrome (Meglič et al., 2008) (Fig. 2).

3.4. The acquired lupus anticoagulant–hypoprothrombinemia syndrome

Although the presence of LAC confers an increased risk of thrombosis, this antibody has been occasionally associated with a severe bleeding diathesis. This complication, termed ''LAC–hypoprothrombinemia syndrome,'' is usually preceded by a viral infection and has been attributed to the presence of antiprothrombin antibodies that could cause rapid depletion of plasma prothrombin (Vivaldi et al., 1997; Yacobovich et al., 2001).

3.5. Neonatal antiphospholipid syndrome

Neonatal APS is an exceedingly rare clinical entity characterized by neonatal thrombotic disease due to the transplacental passage of maternal aPL (Boffa and Lachassine, 2007). In the pediatric age group, the neonatal period carries the highest risk for thrombosis due to the developmental immaturity of the clotting system and frequent need for medical interventions such as indwelling vascular catheters and intensive support. There is growing evidence that transplacentally transferred aPL may contribute to the pathogenesis of neonatal thrombosis, but are not usually sufficient condition for thrombosis, and other possible inherited (i.e. deficiencies of antithrombin, protein C and protein S, factor V Leiden, prothrombin 20120A gene mutation, polymorphism of MTHFR gene) and acquired (i.e. maternal pre-eclampsia, traumatic delivery, complex congenital heart disease, central vascular catheters, sepsis) thrombophilic risk

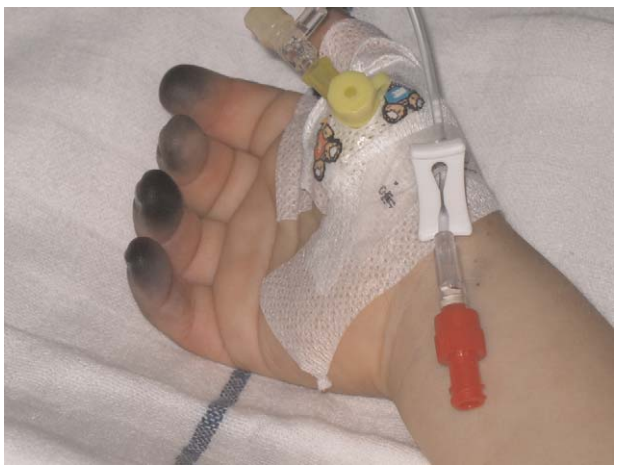

Figure 2. Necrotic changes on the fingertips in a child with microangiopathic antiphospholipid-associated syndrome. (See Colour Plate Section.)

factors should be evaluated systematically (Avčin et al., 2002a; Boffa and Lachassine, 2007).

The most frequently reported association with transplacentally acquired aPL was arterial thrombosis, and ischemic stroke represented half of all the thromboses. Thromboses in other vessels were also described, including aorta, peripheral arteries, mesenteric arteries, renal veins, and subclavian veins (Boffa and Lachassine, 2007). More than 60% of infants with neonatal APS have at least one additional risk factor for thrombosis, most commonly arterial or venous catheters, sepsis, asphyxia, and/or congenital thrombophilia. A recent study evaluated the significance of multiple thrombophilic risk factors in 60 mother–child pairs with perinatal arterial stroke and established positive aCL in 10%, anti-β_2GPI in 25% and LAC in 4% of tested mothers, further supporting the contribution of aPL to perinatal stroke (Curry et al., 2007).

4. Differential diagnosis

Given the spectrum of clinical manifestations, the differential diagnosis of pediatric APS is very broad and depends on target organ involvement. Since aPL-related thrombosis can affect any organ system, pediatricians of different subspecialties must be aware of this syndrome. Characteristic of pediatric thrombosis is the requirement to have multiple risk factors that lead to abnormal clotting (Richardson et al., 2002), therefore, all children presenting with aPL-related thrombotic events should receive a broad investigation for congenital prothrombotic states (protein S, protein C, antithrombin, factor V Leiden, prothrombin 20210 A gene mutation, hyperhomocysteinemia, elevated lipoprotein (a), polymorphism of MTHFR gene) and acquired prothrombotic risks (infection, immobilization, surgery, trauma, dehydration, malignancy, congenital heart disease, nephrotic syndrome, systemic vasculitis, catheter placement).

Differentiation of isolated aPL-related thrombocytopenia from classic idiopathic thrombocytopenic purpura is important to indicate closer follow-up because of risk for the development of future thrombosis or progression to SLE (Diz-Küç ükkaya et al., 2001; Gattorno et al., 2003).

Catastrophic APS should be distinguished from severe lupus vasculitis, sepsis, thrombotic thrombocytopenic purpura, macrophage activation syndrome and disseminated intravascular coagulation (Ravelli and Martini, 2007).

5. Treatment and outcome

Treatment of pediatric patients with aPL is problematic because of the clinical complexity of the syndrome, different pathogenic potential of aPL subtypes and lack of well-designed prospective studies (Lim et al., 2006; Kamat et al., 2006). Modified adult recommendations remain the primary guide for treatment of aPL-related thromboses in pediatric patients, but several differences specific to children must be considered, such as different concentrations of plasma procoagulant and anticoagulant proteins, difficulty of maintaining the appropriate INR levels in infants, anticoagulant side-effects in growing children, a higher risk of hemorrhage during play and sports activities, as well as compliance issues (Monagle et al., 2001). The risk of future thrombosis is low in asymptomatic children who are incidentally found to have positive aPL, high among those in whom thrombosis already occurred, and extremely high in patients with catastrophic APS. A high titer of aPL, in particular the presence of LAC, increases the risk of thrombosis, as do the concomitant presence of inherited prothrombotic disorders and/or other acquired thrombotic risk factors (Male et al., 2001; Kamat et al., 2006). Of note, 21% of children included in the Ped-APS Registry who were initially diagnosed with primary APS over time progressed into a clear-cut SLE or lupus-like disease (Avčin et al., 2008b), which is almost three times higher than that found in adult patients with primary APS (Tarr et al., 2007).

Asymptomatic children, in whom aPL were incidentally found, only rarely develop thrombotic

complications and it is generally assumed that these children do not need any prophylactic treatment. There is considerable controversy as to whether prophylactic treatment is indicated in children who have never had thrombosis but have persistently positive aPL. An expert panel has recommended the use of low-dose aspirin (75–100 mg daily) for prevention of thrombosis in asymptomatic adult patients with a persistently positive aPL (Alarcon-Segovia et al., 2003), however, this recommendation was recently challenged by a prospective, randomized, controlled study which showed that these individuals do not benefit from low-dose aspirin for primary thrombosis prophylaxis (Erkan et al., 2007). The risk of bleeding during play and sports in children might therefore outweigh the possible but unproven benefit.

The risk of thrombosis is higher when additional prothrombotic risk factors are present and prophylaxis with heparin administered subcutaneously may be considered in children with persistently highly positive aPL to cover high-risk situations, such as prolonged immobilization or surgery. Hydroxychloroquine, which has modest anticoagulant properties, may be protective against the development of thrombosis in aPL-positive patients with SLE (Petri, 1996). Aspirin at low dose has also been used as prophylaxis in aPL-positive children with autoimmune diseases and in particular to prevent arterial thromboses, although there is no evidence yet that aspirin may provide protection against thrombosis. The optimal management of adolescents with aPL should also include the avoidance or reduction of other risk factors for thrombosis such as smoking, obesity, high blood pressure, and use of oral contraceptives.

Treatment of the acute thrombotic event in children with APS is no different from that of thrombosis arising from other causes. Most children do receive anticoagulation therapy at the time of diagnosis, but type and duration of therapy are variable. Patients included in the Ped-APS Registry were treated according to the decision of their physician and all patients with venous thrombosis received long-term anticoagulation, but only 40% of patients with arterial thrombosis received anticoagulation therapy with or without concomitant antiaggregation therapy (Avčin et al., 2008b). In spite of the long-term anticoagulation, 19% of pediatric patients with initial venous thrombosis and 21% of patients with initial arterial thrombosis developed recurrent thrombotic events, which is higher than reported in adult APS patients (3–11%) (Crowther et al., 2003; Finazzi et al., 2005). A high thrombosis recurrence rate (29%) was previously reported in two studies in children with APS (Gattorno et al., 2003; Berkun et al., 2006) and 73% of recurrent thrombotic events evolved after cessation of anticoagulation therapy (Berkun et al., 2006). A recent meta-analysis of secondary thromboprophylaxis in adult patients with definite APS suggests prolonged anticoagulation at a target INR of 2.0–3.0 in patients with first venous events and above 3.0 for those with recurrent and/or arterial events (Ruiz-Irastorza et al., 2007). Another large study has demonstrated that either aspirin or moderate intensity warfarin is acceptable for adult patients with aPL and a first episode of ischemic stroke (Levine et al., 2004). Given the high recurrence rate of thrombosis, it seems reasonable to consider anticoagulation in all pediatric patients with definite APS at least at a target INR suggested for adult population. In the study by Levy et al. (2003) in pediatric SLE patients none of the LAC-positive patients who were maintained in the target INR range of 2.0–3.0 developed a second thrombotic event. In the absence of controlled trials, the optimal type and duration of treatment cannot be determined. An improved understanding of the pathogenic mechanisms by which aPL induce thrombosis has also suggested some innovative treatments such as new anticoagulant and antiplatelet drugs, complement inhibitors and monoclonal antibodies (Erkan and Lockshin, 2006); however, there are no available data in the pediatric population.

The risk of recurrent thrombosis is extremely high in patients with catastrophic APS, therefore, their treatment must be complex and include elimination of possible precipitating factors, treatment of the ongoing thrombotic events and suppression of the excessive cytokine storm (Asherson et al., 2003; Erkan et al. 2003; Cervera

et al., 2005). Treatment of known precipitating factors includes prompt use of antibiotics if infection is suspected, excision of necrotic tissues and aggressive treatment with steroid pulses and cyclophosphamide if in the presence of an SLE flare. Treatment of the ongoing thrombosis needs to be aggressive with full doses of heparin and attempts to achieve a rapid reduction of aPL titers by intravenous immunoglobulin or, alternatively, plasma exchange. Other reported management options include fibrinolytic agents, prostacyclin, defibrotide, cyclosporine, azathioprine, rituximab, and hemodialysis.

There are no uniform guidelines for the therapeutic approach in infants with neonatal APS (Boffa and Lachassine, 2007). Infants with stroke usually received only symptomatic treatment for the seizures, with or without antiaggregation, and infants with venous or disseminated thrombotic events received additional anticoagulation, thrombolytic therapy, and/or exchange transfusion. A recent study reported that children born to mothers with APS may exhibit a higher percentage of neurodevelopmental changes, particularly learning disabilities, and it is recommended to include regular neuropsychological assessments during their long-term follow-up (Nacinovich et al., 2008).

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CHAPTER 11

Antiphospholipid Antibodies, Infections, and Drugs

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1. Introduction

In 1906, Wasserman developed the complement fixation assay for the serological diagnosis of syphilis (caused by *Treponema pallidum*), later named the "Wasserman test." This subsequently led to the development of the VDRL (Venereal Disease Research Laboratory) test. Thirty-six years later, Pangborn (1942), isolated a substance from a bovine heart, which she called "cardiolipin" and claimed it to be the pure syphilis antigen. Over the years, lupus patients showed a false-positive VDRL serology, with elevated titers of anticardiolipin antibodies (aCL) in the sera. The presence of aCL was associated with a triad of clinical manifestations: arterial and venous thrombosis, recurrent fetal loss, and thrombocytopenia, which was defined as the anticardiolipin syndrome or "Hughes' syndrome" (Hughes et al., 1986). The renaming to antiphospholipid syndrome (APS) was based on the presence of circulating antiphospholipid antibodies (aPL) in addition to aCL, such as lupus anticoagulant (LAC) with antiprothrombin activity (Asherson et al., 1989a, b).

APS can be primary or secondary to other autoimmune states, mainly systemic lupus erythematosus (SLE) (Cervera et al., 2002). To date, the term "systemic APS" is used for additional organ involvement including cardiac, CNS, cutaneous, renal, hepatic, and other systems (Cervera et al., 2002; Shoenfeld, 2003). In the case of multi-organ failure it is defined as catastrophic APS (CAPS) (Asherson et al., 2003). aPL antibodies may also be detected in infections (Uthman and Gharavi, 2002; Zandman-Goddard et al., 2002; Asherson and Cervera, 2003; Blank et al., 2004; Avcin and Toplak, 2007; Sène et al., 2008; Amin, 2008), diverse vaccinations (Zandman-Goddard et al., 2002; Martinuc-Porobic et al., 2005; Tarján et al., 2006), stem cell transplantations (Barnabe et al., 2009), and tumors (Gómez-Puerta et al., 2006; Pham and Shen, 2008).

The common denominator for all the systemic features in APS is the association with the presence of aPL, directed mainly to the β_2 glycoprotein I (β_2GPI) molecule (Galli et al., 1990). The human β_2GPI molecule is a heavily glycosylated membrane adhesion glycoprotein (326 amino acids), present in blood plasma at a concentration of $\sim$150–300 μg/mL (Bouma et al., 1999). In the current review we will focus on recent data about the link between antiphospholipid/anti-β_2GPI antibodies, infections (viral, bacterial, yeast, rickettsial, spirochetal, parasital), and drugs.

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2. Antiphospholipid antibodies associated with infections

2.1. Viral infections

One of the first studies provided insight into the aCL profile in 23 patients with malaria, infectious mononucleosis, tuberculosis, hepatitis A, or syphilis whose sera did not require the presence of β_2GPI to bind cardiolipin (Hunt et al., 1992). No clinical association to APS was documented in this cohort of patients. A later comprehensive review of the relationship between viral infections and the induction of aPL antibodies between 1968 and 2000 revealed that aCL and/or LAC were associated with a number of viral infections, including hepatitis C virus, human immunodeficiency virus (HIV), cytomegalovirus (CMV), varicella zoster, Epstein–Barr virus (EBV), adenovirus, and parvovirus B19 (Uthman and Gharavi, 2002). Enhanced titers of antibodies to EBV-early antigen of the IgG isotype was found in patients with primary APS (Barzili et al., 2007).

In many instances, the presence of these antibodies to viral antigens was associated with thrombosis (summarized in Table 1). In some patients, the antibodies may be transient and disappear within 2 or 3 months. In other susceptible individuals, they may persist, which raises the question of whether infections may trigger the development of aPL antibodies in autoimmune diseases. The presence of transient LAC and phosphatidylserine-dependent antiprothrombin (aPS/PT) antibodies was described in healthy children who had multiple ecchymoses several days after acute infection (Mizumoto et al., 2006). In another case report, transient elevated levels of aCL antibodies, as a result of infection with herpesvirus-6, was followed by cerebral infarction (Toyoshima et al., 2007). Additional transient expression of aPL antibodies following EBV infection resulted in episodes of deep vein thrombosis (DVT) and pulmonary embolism (Uthman and Gharavi, 2002; vanHal et al., 2005). The transient expression of aPL antibodies in some of the patients with diverse viral infection excludes the presence of long-living latently viral EBV-infected B cells, which may be perturbated upon exposure to a second signal (McNally et al., 1995). In summary, in patients with viral infections, the presence of aPL antibodies is transient in most cases, and is not followed by APS clinical manifestations such as thrombosis. In contrast, the presence of pathogenic anti-β_2GPI antibodies is linked to thrombosis and other APS clinical afflictions.

2.2. Bacterial, mycobacterial, yeast, and parasitic infections and antiphospholipid

The frequency of aPL antibodies associated with infectious agents is summarized in Table 2. In the

Table 1
Antiphospholipid syndrome manifestations associated with viral infections

Infectious agent	Cardiolipin	β_2GPI	Transient	APS manifestations
Hepatitis C	IgG	+	+/−	Thrombosis, brain infarction
Hepatitis B	IgG	−	+	non
EBV	IgG, IgM	+	+	PE, thrombosis
Influenza	IgG	+	+/−	non
Varicella	IgG, IgM	−	+	PE, thrombosis
Parvovirus B19	IgG	+	+	Thrombosis
CMV	IgG, IgM	+	+	Thrombosis
HTLV-1	IgA	−	+	ND
HIV	IgG, IgM, IgA	+	+/−	Leg ulcer necrosis, PE, VE, arterial and vein thrombosis, vasculitis, livedo reticularis
Adenovirus	IgG	+	+	Thrombocytopenia
Herpesvirus-6	IgG	−	+	Cerebral infarct

Note: β_2GPI, β_2 glycoprotein I; PE, pulmonary embolus; ND, not detected; VE, venous thromboembolism.

Table 2
Prevalence of anticardiolipin antibodies in bacterial, rickettsial, yeast, parasitic, and spirochetal infections

Infection/organism	Frequency (%)	aCL isotype
Leprosy	33–67	IgG, IgM, IgA
TB	27–53	IgG, IgM
Bacterial endocarditis	5–44	IgG, IgM
Helicobacter pylori	ND	IgG, IgM
Mycoplasma pneumonia	20–53	IgG, IgM, IgA
S. aureus	43	IgG, IgM, IgA
Streptococcus	80	IgG, IgM, IgA
Streptococcus pyogenes	0–80	IgG, IgM
Salmonella	60	IgG, IgM, IgA
E. coli	67	IgG, IgM, IgA
Ornithosis	33	IgG, IgM, IgA
Coxiella burnettii	42–84	IgG, IgM
Leptospirosis	50	IgG
Borrelia burgdorferi	14–41	IgG, IgM
Saccharomyces cerevisiae	ND	IgG
Malaria	30	IgG, IgM
Kalaazar	ND	IgG

Note: ND, not defined.

context of viral infections the prevalent circulating aPL are the aCL non-aPL antibody is the aCL non-β_2GPI-dependent. Anticardiolipin antibodies from patients with infection, unlike those from patients with SLE, APS, do not have the β_2GPI requirements. Serum from 114 patients with infections including syphilis ($n = 11$), tuberculosis ($n = 63$), and *Klebsiella* ($n = 42$) were assayed for β_2GPI and aCL antibodies. The incidence of aCL in serum of patients with tuberculosis. *Klebsiella* infection, and syphilis was 6.0%, 5.0%, and 64.0%, respectively, but all patients were negative for β_2GPI . The results indicate that the β_2GPI is negative in patients with transiently positive aCL associated with infection. Nevertheless, in patients with leprosies we have found three subpopulations of aPL in the sera of these patients (Hojnik et al., 1994). Increased levels of IgG aCL/β_2GPI complex were detected in the sera of 59 out of 61 leprosy patients. IgG aCL was demonstrated in 10 out of 31 leprosy sera and 9 out of 10 APS sera. In 11 out of 61 leprosy sera increased levels of anti-β_2GPI IgG antibodies were found. Others have found increased titers of circulating anti-β_2GPI IgM (deLarrañaga et al., 2000), or aCL associated with disease activity (Elbeialy et al., 2000). Interestingly, a direct link between *Helicobacter pylori* and aPL was shown in a patient with catastrophic APS with high titers of aCL antibodies; these levels decreased following *H. pylori* eradication (Cicconi et al., 2001).

The possibility of a relationship between *Saccharomyces cerevisiae* and anti-β_2GPI antibodies intriguing. The presence of circulating anti-*Saccharomyces cerevisiae* IgA/IgG antibodies is one of the main markers for Crohn's disease, an idiopathic chronic inflammatory bowel disease (Shafran et al., 2002). Patients with inflammatory bowel diseases also have elevated titers of circulating aCL/anti-β_2GPI antibodies (Koutroubakis et al., 1998; Aichbichler et al., 1999; Shafran et al., 2002; Thong et al., 2002). Episodes of thrombosis associated with Crohn's disease have been described, although the association with elevated titers of anti-β_2GPI is still not clear (Koutroubakis et al., 1998; Aichbichler et al., 1999; Thong et al., 2002).

We have evaluated the prevalence and properties of anti-*Saccaromyces cerevisiae* antibodies (ASCA) in APS patients (Krause et al., 2007). Thirty-one out of 155 APS patients tested positive for ASCA (20.0%), compared with 5.0% in healthy controls ($p < 0.05$). The presence of ASCA was not associated with any specific manifestation of APS. The ASCA found to be the population of anti-β_2GPI. Affinity-purified anti-β_2GPI from ASCA-positive sera on a β_2GPI column specifically bound the phosphopeptidomannan (PPM) part of the cell wall of the yeast, as shown by direct binding and competition assays (95–98%). The PPM differentially inhibited anti-β_2GPI binding to β_2GPI. Since the anti-β_2GPI anti-PPM could bind only the native form of β_2GPI and not the recombinant form, we assumed that these specific anti-β_2GPI subpopulations of antibodies are directed to the glycosylated site of the molecule.

Cross-reactive antibodies between a pathogen (*Streptoccocus pyogenes*) and anti-β_2GPI antibody has been demonstrated by us in the case of rheumatic fever. Rheumatic fever is a classical example of molecular mimicry between the streptococcal M protein pathogen and cardiac tissue antigens, leading to cardiac destruction; similarly the mimicry between *N*-acetyl-beta-D-glucosamine (GlcNAc) and neuronal cells causes Sydenham's chorea (Blank et al., 2006). Rheumatic fever and APS are

autoimmune diseases that share similar cardiac and neurological pathologies. We assessed the presence of shared epitopes between M protein, GlcNAc, and β_2GPI. Affinity-purified anti-β_2GPI antibodies were found in 24.4% of 90 patients with rheumatic fever. Anti-β_2GPI antibodies from patients with APS bound streptococcal M protein and its synthetic derivatives, as well as GlcNAc. The binding of anti-β_2GPI antibodies to GlcNAc was confirmed by exposure of the autoantibodies to a glycan library on a chip (data not published). These results proposed an overlap of humoral immunity in rheumatic fever and APS, supporting the hypothesis that common pathogenic mechanisms underlie the development of cardiac valve lesions and CNS abnormalities in both diseases (Blank et al., 2006).

N-Linked glycans, such as GlcNAc and galactose-*N*-acetylgalactosamine (GalNAc), are among the diverse molecules on the bacterial cell wall. We screened sera from 72 APS patients, and used ELISA for binding to a profile of various glycans (not published). A significantly high level of anti-GlcNAc-beta, GalNAc-beta, GalNAc-alpha, Neu5Ac-alpha, and beta4GlcNAc-beta IgG were found in APS patients versus normal controls. These differences between antibody levels enable us to distinguish between APS patients and normal controls with high sensitivity ($\sim$80%) and specificity ($\sim$80%). Between 40% and 60% of anti-glycan antibodies in APS patients were cross-reactive with β_2GPI, which is a heavily glycosylated molecule.

Recurrent fetal loss is one of the clinical characteristics of APS. Elevated anti-GalNAc-beta IgG levels were significantly associated with pregnancy loss vs. non-pregnancy loss with 56% sensitivity and 85% specificity ($p = 0.02$). In a cohort of 95 APS patients (46 with thrombosis and 49 without thrombosis), no significance difference was found between levels of anti-glycan antibodies. Among the 52/95 APS patients with CNS involvement (stroke, epilepsy, or migraine), no significant difference was found between levels of anti-glycan antibodies and CNS events. The above data provided evidence that aPL antibodies contain significant levels of anti-glycan antibodies directed to GlcNAc-beta, GalNAc-beta, GalNAc-alpha, Gal-beta4GlcNAc-beta IgG and anti-GalNAc-beta IgG may have prognostic importance for predicting pregnancy loss.

3. The infection origin of the antiphospholipid syndrome

The association between clinical manifestations of APS, such as thrombosis and pulmonary emboli, following diverse infections in the past is described in Tables 1 and 2 and has been reviewed extensively (Uthman and Gharavi, 2002; Zandman-Goddard, 2002; Asherson and Cervera, 2003; Blank et al., 2004; Avcin and Toplak, 2007; Sène et al., 2008; Amin, 2008). A decade ago, we (Blank et al., 1999a, b, 2002) and others (Gharavi et al., 2002; Pierangeli et al., 2004) proposed that molecular mimicry between infectious agent and the β_2GPI molecule may lead to the generation of pathogenic anti-β_2GPI antibodies and we hypothesized that it may be one of the etiologies of APS.

When we were looking for a main target epitope on the β_2GPI molecule for anti-β_2GPI antibodies affinity purified from patients with APS, we introduced the studied autoantibodies to a peptide phage-display library. Following bio-panning procedures, we identified three consensus amino acid sequences (Blank et al., 1999a, b). The three peptides (A, NTLKTPRVGGC; B, KDKATFGTHDGC; and C, CATLRVYKGG) were found to be mimetics of three different regions on the β_2GPI, corresponding to domains I–II, III, and IV of the β_2GPI molecule (Blank et al., 1999a, b). The β_2GPI-related peptides differentially ameliorated the anti-β_2GPI-mediated-endothelial cell activation, inhibited adhesion of monocytes to the endothelial cells and expression of adhesion molecules in vitro. In an animal model, the peptides prevented the induction of experimental APS by anti-β_2GPI antibodies in naïve mice. The SwissProt protein database revealed high homology (complete or one mismatch) between the new hexapeptides that bind to the anti-β_2GPI antibodies and the membrane particles of different bacteria and viruses. The sequence LKTPRV showed homology to eight different bacteria (such as *Pseudomonas aeruginosa*) and homologies to five

types of viruses including polyomavirus, human cytomegalovirus (CMV), and adenovirus. The sequence TLRVYK showed homology to eight different bacteria, including *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Shigella dysenteriae*, and to viruses such as Epstein–Barr virus (EBV) and human immunodeficiency virus (HIV) (Blank et al., 1999a, b). In order to prove possible molecular mimicry between the pathogen and the β_2GPI molecule, we had to prove the the pathogen is able to induce pathogenic anti-β_2GPI antibodies in an experimental model. Thus, BALB/c mice were immunized with microbial pathogens that share structural homology with the TLRVYK peptide. The mice developed anti-β_2GPI antibodies with different affinities to the β_2GPI molecule. The highest anti-β_2GPI affinity was developed in mice immunized with *Haemophilus influenzae*, *Neisseria gonorrhoeae* or tetanus toxoid (Blank et al., 2002). The sera, from the specific pathogen-immunized mice, was loaded on a relevant β_2GPI-related synthetic peptide coupled to sepharose. The eluted anti-β_2GPI-related synthetic peptide antibodies were infused intravenously into naïve mice (Blank et al., 2002). The mice infused with anti-TLRVYK antibodies developed experimental APS manifested by thrombocytopenia, prolonged activated partial thromboplastin time (aPTT), and increased fetal loss, similar to a control group of mice infused with pathogenic anti-β_2GPI antibodies. Moreover, the synthetic peptide A: NTLKTPRVGGC, that shares similarity with common bacterial antigens (Blank et al., 1999a, b), was able to reverse aPL-mediated thrombosis in mice in vivo (Pierangeli et al., 2004). APL antibodies may be induced in mice by immunization with a CMV-derived peptide causing thrombosis and activation of endothelial cells in vivo (Gharavi et al., 2002; Pierangeli et al., 2004). Peptides which harbor homology to various infectious agents such as CMV, human adenovirus type 2, *Bacillus subtilis*, and a putative phospholipid-binding region on the β_2GPI, induce an experimental APS mouse model (Gharavi et al., 1999). However, in another thrombosis model, infusion of total IgG with anti-β_2GPI antibody activity into rats did not cause thrombosis unless bacterial lipopolysaccharides (LPS) as a "second hit" was given to the rats (Fischetti et al., 2005).

Endothelial cell signaling mediated by anti-β_2GPI or LPS was analyzed, employing transient co-transfected human microvascular endothelial cells with dominant-negative constructs of different components of the pathway (Delta TRAF2, Delta TRAF6, Delta MyD88) together with reporter genes (NF-κB luciferase and pCMV-beta-galactosidase) (Raschi et al., 2003). The results showed that there is a similarity in the signaling cascade induced by anti-β_2GPI antibodies and LPS or interleukin-1 (IL-1). Delta TRAF6 and Delta MyD88 significantly abrogate antibody-induced as well as IL-1 or LPS-induced NF-κB activation. Delta TRAF2 (involved in NF-κB activation by tumor necrosis factor) does not affect the activation, leading to the possibility of an involvement of the toll-like receptor (TLR) family on the endothelial cell surface, and direct induction of activation leading to a procoagulation state (Raschi et al., 2003). LPS activates systemic inflammatory responses through TLR4. Many studies have suggested that TLR4 expressed on B cells recognizes LPS and some pathogenic antigens, including viral proteins and parasitic heat shock proteins (Peng, 2005). Pierangeli et al. (2007) proved the importance of TLR4 in the pathogenic process in APS by studying the thrombogenic aPL activity in LPS non-responsive ($LPS^{-/-}$) mice and the association between TLR4 gene polymorphisms and APS in patients. IgG affinity purified from patients with APS induced more severe thrombi formation and enhanced leukocyte adhesion to endothelial cells in the cremaster muscle microcirculation of $LPS^{+/+}$ mice than control human IgG used in the study. aPL depleted of β_2GPI induced significantly smaller thrombi and fewer leukocytes adhering to endothelial cells in $LPS^{-/-}$ mice than in $LPS^{+/+}$ mice. IgG APS induced higher tissue factor (TF) activity in carotid artery homogenates of $LPS^{+/+}$ mice than in $LPS^{-/-}$ mice. The prevalence of Asp299Gly and Thr399Ile *tlr4* polymorphisms was significantly lower than in controls.

Based on the above data in $LPS^{-/-}$ mice, and the reduction in the protective polymorphism in patients with APS with thrombosis, it is suggested that TLR4 is involved in the interaction of aPL with endothelial cells in vivo (Pierangeli et al., 2007).

4. Antiphospholipid and vaccination

Antiphospholipid antibodies were found in the sera of healthy individuals following vaccinations. In some cases, thrombotic events were encountered. Vaccination of a population of healthy students (63 female, 22 male; mean age 20.8 years) with recombinant hepatitis B (HBV) resulted in elevated titers of aCL antibodies (IgM and IgG isotypes) (Martinuc-Porobic et al., 2005). One month after vaccination with the first dose of HBV a minority of vaccinated individuals showed changes in IgG or IgM aCL or anti-β_2GPI or LAC activity ($p<0.001$). Among subjects in whom changes of IgG anti-β_2GPI were observed, a significantly higher number of elevated titers (8/85) compared with decreased titer (2/85) values were found ($p<0.01$). This study proved that HBV can induce aPL, although rarely. In genetically susceptible individuals, or together with some other triggers, such a combination might confer the risk of developing a continuous autoimmune response.

A six-month follow-up after an annual influenza vaccination in a population of 92 healthy adults was conducted in Slovenia (Toplak et al., 2008). Sixteen per cent developed aCL antibodies, 7% anti-β_2GPI antibodies, with no statistical difference between both autoantibodies and LAC one month and six months post vaccination. Two participants showed progressive increases in aCL IgM or anti-β_2GPI IgA. Eleven individuals had a transient increase in the autoantibodies (Toplak et al., 2008). Elevated aPL titers were shown also in patients with SLE (Tarján et al., 2006). A case was reported in which an ischemic stroke event occurred in a patient with lupus following influenza vaccine (Vainer-Mossei et al., 2009). Additional cases of generation of aCL antibodies following vaccination with influenza was reported in Henoch–Schönlein purpura vasculitis. Four months following convalescence, IgG aCL were positive while C3 was slightly elevated without any clinical symptoms in the patients (Mormile et al., 2004).

5. Drug-induced antiphospholipid antibodies

Some drugs may induce generation of aPL antibodies, as in drug-induced lupus. The prevalence and pathogenicity of drug-induced aPL is generally low. The known drugs that induce lupus, such as procainamide, phenothiazines, quinine, and oral contraceptives, may induce aPL antibodies. The mechanisms leading to disease development may differ among the various drugs. Procainamide and some other drugs induce aPL associated with thrombosis (Li et al., 1988; Schlesinger and Peterson, 1988; Asherson et al., 1989a, b); phenothiazine-induced aPL are considered fairly benign; while chlorpromazine is the most common medication associated with drug-induced aPL (Canoso and de Oliveira, 1988). In some cases, the clinical manifestations of drug-induced aPL antibodies include thrombosis, abortion, and cerebrovascular disease.

Susceptibility to the induction of drug-induced aPL may depend on various factors: (a) acetylator status (slow acetylators develop antibodies earlier than fast acetylators); (b) HLA DR status (a high incidence of HLD DR4 has been found in hydralazine-associated SLE); and (c) the existence of null alleles at the C4A and CB4 loci (ir4) and inhibition of binding of C4 to activated C1S (Sim et al., 1984; Spiers et al., 1989).

One case report has shown the development of lupus-like syndrome and significantly elevated IgG and mildly elevated IgM aCL titers in a 30-year-old woman treated with quinine for malaria (Rosa-Re et al., 1996). Once quinine was withdrawn, there was a prompt relief of the symptoms and the laboratory tests returned to normal shortly thereafter.

Treatment of rheumatoid arthritis with infliximab (Remicade) has been associated with the induction of antinuclear autoantibodies (ANA) and anti-dsDNA antibodies. Investigating the humoral immune response induced by infliximab against organ-specific or non-organ-specific antigens in rheumatoid arthritis and in patients with ankylosing spondylitis during a 2-year follow-up revealed that the incidence of aPL antibodies was significantly higher in both rheumatoid arthritis patients (21%) and ankylosing spondylitis patients (27%) than in the control group (Ferraro-Peyret et al., 2004; Nosbaum et al., 2007). Most anti-dsDNA and aPL antibodies were of the IgM isotype and were not associated with infusion side-effects, lupus-like manifestations, or infectious disease.

6. Summary

The association between the generation of aPL antibodies and infectious agents is well established. In most cases, the generation of aPL antibodies seems to be transient. In some cases, the presence of aPL antibodies is linked to thrombotic events, recurrent fetal loss, or other clinical manifestations of APS. The question is when does the presence of the aPL antibodies lead to a pathophysiological state? Several explanations have been put forward: (a) There may be a genetic predisposition to an autoimmune condition. (b) A specific amino acid sequence in the complementarity-determining regions (CDRs) of the immunoglobulin structure may cause the antibody to be pathogenic (Blank et al., 1999a, b). Previously we have proven that exchanging heavy and light chains between a pathogenic and non-pathogenic aPL resulted in a change in the activity of the immunoglobulin. The pathogenic aPL, having the heavy chain from the non-pathogenic, lost its ability to induce experimental APS and vice versa (Blank et al., 1999a, b). (c) The aPL antibody may recognize a self structure by molecular mimicry in concert with a pro-inflammatory micro/macroenvironment. (d) There may be explosion of a self-reacting B cell clone due to a bystander activation process of a latently infected memory cell triggered by a second hit (infection, inflammation, or trauma) associated with a decrease in T-regulatory cells feedback mechanism. This combination may result in generation of aPL antibodies.

Finally, in infections (with or without association with a second hit) production of aPL as a result of combination of the above described possibilities may explain the generation of pathogenic, non-pathogenic, or transient circulating aPL antibodies.

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CHAPTER 12

Antiphospholipid Syndrome Associated with Malignancies

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1. Introduction

The antiphospholipid syndrome (APS) is an acquired prothrombotic disorder characterized by the presence of arterial or venous thrombosis and pregnancy loss. Although the early description by Hughes (1983), almost 25 years ago, was in patients affected by SLE, during recent years, the clinical spectrum of APS has extended to other fields, recognizing the presence of antiphospholipid antibodies (aPL) in a series of other conditions such as systemic chronic infections, other autoimmune diseases (i.e. systemic vasculitis), and malignancies among others.

Since the discovery of anticardiolipin (aCL), there have been many isolated case reports of the association of aCL with vascular events in patients with a variety of malignant conditions, including solid tumors, and lymphoproliferative and hematological malignancies. It is now clear that aPL should always be considered in the pathogenesis of vascular occlusion occurring in patients demonstrating Trousseau's syndrome (Asherson, 2000).

Trousseau (1865) first drew attention to thrombotic occlusions in patients with carcinoma and a variety of pathogenic factors have been implicated in the association. There has been experimental work demonstrating tumor growth with agents activating blood coagulation and regression with coagulation inhibitors. Fibrin generation has also been associated with accelerated tumor growth and tumor cells themselves may be responsible for the production of compounds resulting in this mechanism of thrombosis (Asherson, 2000).

Several mechanisms have been suggested for the association between aPL and cancer including the following: (1) production of autoantibodies by the immune system as a response to tumor antigens; (2) production of monoclonal immunoglobulins with lupus anticoagulant (LAC) and aCL activities; and (3) secretion of aCL from tumor cells (Gómez-Puerta et al., 2006).

2. Solid and hematological malignancies and antiphospholipid antibodies

Some studies have focused on the association between aPL and solid and hematological malignancies (Tables 1 and 2) but with limited information on their clinical (thrombotic) presentation. A large prospective epidemiological study on the occurrence of malignant disease in aPL-positive patients was conducted in Montpelier, France in 1994 (Schved et al., 1994). In this study 1014 patients were tested at entry and, interestingly, carcinoma was the most frequently associated disease. Of the 72 aPL-positive patients, 14 had a history of carcinoma, 9 had active malignant disease while 5 were in clinical remission. The

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Table 1
Hematological malignancies associated with antiphospholipid antibodies

Type	No. (%)
B-cell lymphoma	10 (8)
Spleen lymphoma	8 (7)
Chronic myeloid leukemia	7 (6)
Non-Hodgkin's lymphoma	6 (5)
Lymphocytic lymphoma	4 (3)
Hairy-cell leukemia	4 (3)
Cutaneous T-cell lymphoma	3 (3)
Multiple myeloma	3 (3)
Hodgkin's disease	3 (3)
Acute myeloid leukemia	2 (2)
Chronic lymphoid leukemia	2 (2)
Lymphosarcoma	2 (2)
Peripheral T-cell lymphoma	1 (1)
Waldenström macroglobulinemia	1 (1)
Acute lymphoid leukemia	1 (1)
Monoclonal gammopathy	1 (1)
Myeloproliferative syndrome	1 (1)
Acute monocytic leukemia	1 (1)
Angiocentric lymphoma	1 (1)
Lymphoplasmacytoid lymphoma	1 (1)

From Gómez-Puerta et al. (2006).

Table 2
Solid organ malignancies associated with antiphospholipid antibodies

Type	No. (%)
Renal cell carcinoma	7 (6)
Primary tumor with unknown origin	6 (5)
Lung adenocarcinoma	6 (5)
Breast carcinoma	6 (5)
Melanoma	6 (5)
Prostatic adenocarcinoma	4 (3)
Otorhinolaryngology tumors	3 (3)
Non-small cell lung cancer	3 (3)
Central nervous system tumors	2 (2)
Uterine carcinoma	2 (2)
Colon carcinoma	2 (2)
Ovarian carcinoma	2 (2)
Cholangiocarcinoma	2 (2)
Leiomyoblastoma	1 (1)
Thymoma	1 (1)
Hepatocarcinoma	1 (1)
Mesothelioma	1 (1)
Tracheal carcinoma	1 (1)
Gastric carcinoma	1 (1)
Carcinoid tumor	1 (1)
Papillary thyroid carcinoma	1 (1)
Leiomyosarcoma	1 (1)

From Gómez-Puerta et al. (2006).

main related malignancies found were prostatic adenocarcinoma, breast carcinoma, ovarian carcinoma, and colon adenocarcinoma.

Zuckerman et al. (1995) studied the prevalence of aCL in patients with malignancy and the possible association of aCL with thromboembolic events. They included 216 patients in their group and an age-matched control group of 88 healthy subjects. Forty-seven (22%) of the cancer patients were found to be aCL-positive compared with only three (3%) of the control group. The aCL-positive cancer patients had a significantly higher rate of thromboembolic events than aCL-negative cancer patients ($13/47 = 28\%$) vs. ($24/169 = 14\%$) respectively ($p < 0.05$).

Miesbach et al. (2006) retrospectively studied the thrombotic manifestations in 58 patients demonstrating aPL and a history of neoplasia. Thirty-nine patients (67%) suffered from solid tumors such as tumors of the breast in 9 patients, prostate in 4, urinary tract in 4, colon in 4, brain in 3, thyroid in 3, larynx in 3, kidney in 2, cervix in 2, skin in 2, tonsils in 1, cutaneous squamous cell carcinoma in 1, parotid in 1, testicle in 1, and liver tumor in 1 patient. Nineteen patients (33%) had hematological or lymphoproliferative malignancies, including non-Hodgkin's lymphoma in 9 patients, myeloproliferative disease in 5 patients, acute leukemia in 2 patients, Waldenström's macroglobulinemia in 2 patients and monoclonal gammopathy in 1 patient. Four patients suffered from a combination of malignancies such as carcinoma of the breast and hypophysis, carcinoma of the breast and melanoma, carcinoma of the kidney and testis, malignant lymphoma with a carcinoma of prostate and testis.

Of the 58 patients, 46% had positive LAC, 41% had elevated IgG aCL, 64% had elevated IgM aCL titers, and 55% had elevated levels of both. Of the patients with solid tumors, 18/39 (46%) patients had thromboembolic complications of the APS. Of the patients with hematologic and lymphoproliferative malignancies, only 6/19 (32%) suffered from thromboembolic complications. There was, however, no relation between the titers of aCL antibodies and the clinical

manifestations. Finally, the authors suggested that the presence, but not the titers, of aPL may identify a subset of cancer patients with a high risk of developing thrombotic complications (Miesbach et al., 2006).

It has previously been pointed out that the development of aPL-related complications is less common in patients with hematological malignancies (Lossos et al., 1998) and is even less common in patients with lymphoproliferative disorders (Asherson, 2000). It is possible that the presence of these antibodies may be the result of the production of abnormal proteins such as monoclonal immunoglobins which accompany monoclonal gammopathy (Lechner and Pabinger-Fasching, 1985). Stimulation of the B cells and consecutive production of a variety of autoantibodies has also been hypothesized.

This was confirmed by a recent study including four patients with elevated IgM aPL titers lying outside the region signifying 95% of normal cases and with a history of non-Hodgkin's lymphoma. The patients had elevated IgG and IgM aCL and also tested positive for LAC. Other aPL were measured, and we found high positive results for all tested antibodies in three patients. The production of aPL, however, occurred in the absence of thrombotic complications. No thromboembolic manifestations occurred during the follow-up period either. It could also be demonstrated that the degree to which the aCL titer was elevated resembles the elevation of the non-classical aPL, but not that of β_2 glycoprotein I (β_2GPI) or anti-annexin antibodies. Therefore, it can be postulated that these extremely high levels of IgM aCL antibodies do not enhance the risk of thrombosis and may be completely different from aCL antibodies in an APS patient population without malignancies. In particular, hematological and lymphoproliferative malignancies may indeed be associated with the generation of aPL, but do not necessarily enhance the thrombophilic risk in these patients (Miesbach et al., 2006).

There are limited data on whether patients with primary APS have an increased risk of developing cancer as occurs in other systemic autoimmune diseases (e.g. systemic lupus erythematosus (SLE), primary Sjögren's syndrome, rheumatoid arthritis, or dermatomyositis) (Naschitz et al., 1999). Finazzi et al. (1996) evaluated 360 patients with aPL (primary APS in 207, SLE in 112) who were followed for 5 years with regular 6-monthly examinations. They reported that after 4 years of follow-up, 4 patients with primary APS developed a malignant disease (1 breast carcinoma and 3 non-Hodgkin's lymphoma (NHL)), resulting in an estimated rate of 0.28% patient/year, a far higher incidence of NHL than its incidence in the general Western population. Five out of 18 patients who died during the follow-up period had developed hematological malignancies.

The pathological significance of aPL in patients with malignancies is, however, still unclear. It has not been established whether the presence of aPL may be considered an "epiphenomenon" of the malignant disease or whether it contributes directly to the development of thrombosis in these patients. In the study by Miesbach and colleagues (2006), over a period of 4 years, a history of malignancy was found in 58 out of 425 aPL-positive patients (14%), confirming that underlying malignancy is an important cause of APS.

In the study of Gómez-Puerta and colleagues (Gómez-Puerta et al., 2006), 29 out of 120 cases of malignancy were diagnosed after the thrombotic manifestation of APS. Since the publication of that series of patients with malignancies and APS, new cases with primary APS who developed a malignancy (one hairy cell leukemia and one Waldenstrom's macroglobulinemia) have been reported (Asherson et al., 2007; Diz-Kucukkaya et al., 2007).

Most of the studies included patients retrospectively, generating the hypothesis that some patients with the previously considered paraneoplastic thrombosis had, in fact, APS. Other important factors, however, such as the stage of the malignancy or the progression at the time of the thrombosis were not considered. Unfortunately, not all patients could be followed up during the years after treatment of the malignancy. In two patients of the study of Miesbach et al., however, aPL disappeared after effective treatment of a carcinoma of the colon. These patients had no further thrombotic events and aPL remained negative. In a reported study of 22 patients with

NHL, more than 40% of the patients with elevated aPL normalized after effective therapy, whereas levels seemed to rise again during a relapse (Sciarra et al., 1995). In another study, aPL were demonstrated in 24 out of 90 NHL patients. Over a median follow-up period of 14 months, none of the aPL-positive patients developed thromboembolic events (Genvresse et al., 2002). As this study was retrospective and the timeline between the diagnosis of malignancy and diagnosis of thrombosis differed in the patients, a prospective study is needed to identify patients with malignancy at the time of their diagnosis to check for aPL over time and to study the clinical manifestations of APS.

In APS associated with autoimmune diseases or chronic infections, aPL titers wax and wane over time, but do not usually disappear. This situation seems different in APS associated with cancer, where, in a substantial number of patients (around one-third), aPL disappear after correct treatment of the malignancy.

Espinosa and colleagues (2008) evaluated the presence of aCL antibodies in a series of 241 patients with cancer seen in a medical Oncology Department and compared them with 120 healthy individuals, age- and gender-matched. One hundred and ninety out of 241 patients had previous venous thrombosis. The most frequent solid tumors were lung (25.7%), colon (19.1%), and breast carcinoma (13.7%). Predisposing clinical factors to thrombosis were found in 45.3% of patients, including immobilization, postoperative state, and intravenous catheter. The prevalence of aPL in cancer patients with thrombosis was significantly elevated compared with cancer patients without thrombosis and with healthy individuals (10% vs. 0% vs. 0%; $p<0.005$). LAC were detected in 9 (4.7%) patients, and the aCL titer was positive in 11 (5.8%). All patients were positive for the IgG aCL (3 at low titers, 4 at moderate, and 4 of them showed titers >40 UGPL). The authors concluded that in comparison with cancer patients without thrombosis and healthy individuals, cancer patients with thrombosis had an elevated prevalence of aPL. In addition it was suggested that the presence of aPL may identify a subset of cancer patients who are at high risk of developing thrombotic complications.

3. Catastrophic antiphospholipid syndrome and malignancies

A particularly serious clinical form of the APS with a mortality rate of approximately 50%, despite treatment, has been termed the catastrophic APS (Asherson's syndrome or CAPS) (Piette et al., 2003). In the majority of cases, these patients present with fulminant thrombotic complications predominantly affecting small vessels of organs. Large vessel occlusions do occur but with a considerably reduced frequency compared with their occurrence in the simple/classic APS. These consist of deep vein thromboses, complicated by pulmonary embolism or major arterial occlusions (e.g. stroke).

Despite our increasing understanding of the underlying mechanisms and clinical manifestations of CAPS, thrombotic complications are nevertheless still unpredictable and "triggering factors" are not identifiable in the majority of cases. Risk factors are increasingly being identified. These include warfarin withdrawal, surgery, or prior infections. One important risk factor for CAPS appears to be a history of malignancy (Asherson, 2000).

There is a website-based international registry of patients with CAPS for all cases in which both CAPS and underlying malignancies are present. The clinical characteristics of these cases were subsequently evaluated to establish common characteristics. The CAPS registry includes information on a total of 262 cases.

Of the 262 cases included in the CAPS registry, 23 (9%) patients had malignancies; 14 (61%) were female and 9 (39%) were male. The mean age was 46.9 years with a standard deviation of 12 years (range 32–71 years). Of the patients, 6 (26%) had an underlying rheumatic disorder. Of these patients, 3 had SLE, 1 had lupus-like disease, 1 had scleroderma and 1 had polymyositis. LAC were detected in 17 patients (74%), IgG aCL in 15 patients (65%), and IgM aCL in 7 patients (30%). Thrombocytopenia was present in 9 patients (39%). Hematological malignancies were present in 6 patients (26%): lymphoma, NHL, acute lymphatic leukemia, angiocentric lymphoma, chronic myelocytic leukemia, and Hodgkin's

lymphoma. The other patients mainly had lung carcinoma (17%). Two (9%) patients had colon carcinoma.

In 78% of these patients, the malignancy itself or the treatment modalities instituted for the carcinoma was the precipitating factor of CAPS. Only 39% of CAPS patients with malignancies recovered in comparison with 58% of patients without malignancies ($p = 0.07$). Treatment modalities, however, did not differ significantly between these patients.

Infections were not evident as precipitating factors for any of the malignancy patients. The mean age of patients with malignancies was 9 years older than the average age of other patients with CAPS and the prevalence of SLE was significantly less common than in patients without malignancy. The survival rate of patients with CAPS is poor and the optimal treatment for patients with CAPS has not yet been established. The outcome of patients with CAPS is worse in the presence of an additional malignancy than when no malignancy is present. Only 39% of CAPS patients with malignancies recovered. This may be due to the additional presence of the malignancy and to the older age of the patients. Other confounding factors were not found.

Treatment modalities did not differ significantly between patients with and without malignancies. Treatment by plasma exchange, however, was used more frequently in patients with malignancies. The poorer survival rate in patients with malignancies might have nothing to do with the treatment modalities (Miesbach et al., 2007a, b).

Malignancy may play a pathogenic role in patients with CAPS, whereas infections are more important as triggering factors in patients without malignancies. CAPS patients with malignancies are generally older than CAPS patients without malignancies; they generally have the worst prognosis of the entire CAPS cohort.

4. Therapeutic aspects

Recent evidence shows that anticoagulant therapy, e.g. low-molecular-weight heparin (LMWH), may have anti-neoplastic effects and may improve survival in patients with malignancy (Hettiarachchi et al., 1999). Patients with APS should be treated with anticoagulants for life. Particularly in APS patients with malignancy, treatment with LMWH may be more effective than conventional oral anticoagulant therapy with coumadin (Meyer et al., 2002). Further studies are needed to investigate the influence of anticoagulants in patients with APS and malignancies.

5. Conclusion

In conclusion, the presence of aPL may contribute to an increased risk of thrombosis in patients with malignancies, although the levels do not seem to reflect their pathogenicity. Conversely, other malignancies, particularly hematological and lymphoproliferative malignancies, may indeed be associated with the generation of aPL but do not necessarily enhance the thrombophilic risk in these patients. In the future, distinguishing the different types of aPL will, no doubt, present an opportunity to learn more about the pathogenic potential of the various aPL, themselves, in a large variety of conditions, including in the presence of malignancies.

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CHAPTER 13

Antiphospholipid Antibodies and Vasculitis

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1. Introduction

The vascular lesions that occur with the antiphospholipid syndrome (APS) (Harris et al., 1987) and which form the basis of the clinical syndrome are associated with a non-inflammatory vasculopathy (Asherson and Cervera, 1992) resulting in recurrent venous and arterial thrombosis, fetal losses as well as livedo reticularis, valvular heart disease, and thrombocytopenia. Many other manifestations have been documented (Alarcón-Segovia et al., 1989), the majority of which are consequent on vascular occlusions, but in others (e.g. chorea and impaired fecundity rate) other mechanisms have been invoked.

It has been proposed by Alarcón-Segovia and Drenkard (2000) that vasculitis associated with antiphospholipid antibodies (aPL) should be defined as: (1) ''probable vasculitis,'' including lesions characteristic of vasculitis that were not biopsied plus palpable purpura, punctate erythematous lesions in the palms or soles, urticarial lesions, mononeuritis multiplex, leg ulcers, or digital necrotic lesions; or (2) ''definite vasculitis,'' where the clinical diagnosis is confirmed histologically and/or by arteriography. Vasculitis is only present when an inflammatory cell infiltrate is present with accompanying destruction and/or fibrinoid necrosis in the vessel walls. Thrombosis may, of course, be superimposed on this vasculitis. Organized thrombus on the other hand may also demonstrate some reactive cellular infiltrate and this may on occasion be misinterpreted as vasculitis. Immune complex deposits do not, by themselves, constitute evidence of vasculitis.

There has been controversy surrounding the question of vasculitis found in the APS, and various authors have attempted to unravel this question in several publications (Lie, 1989, 1994, 1996; Goldberger et al., 1992). Lie defined vasculitis as a true inflammatory disease of the blood vessels, artery, or vein, with demonstrable structural injury to the vessel wall, the cause of which may be unknown (primary vasculitis), or known (secondary vasculitis) (Lie, 1996). Since his seminal work was published no further light has been shed on this topic, however. Vasculitis in association with aPL—either anticardiolipin antibodies (aCL) or lupus anticoagulant (LAC)—has been encountered in many circumstances, notably when patients with systemic lupus erythematosus (SLE) have vasculitic lesions associated primarily with the SLE itself, in which case the vasculitis is occurring with underlying disease and coincidentally aPL are present (Lie, 1996). Vasculitis has also been documented in other related conditions such as the ''primary'' APS (Asherson, 1988; Asherson et al., 1989; Jeffrey et al., 1989; Ames et al., 1992; Lie et al., 1995) (Table 1).

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Table 1
Vasculitic syndromes associated with antiphospholipid antibodies

Cutaneous vasculitis
Non-cutaneous organ-specific vasculitis (large and small vessels)
Systemic vasculitides (PAN, Wegener's granulomatosis, microscopic polyangiitis)
Relapsing polychondritis
"Capillaritis" (pulmonary, renal), superficial thrombophlebitis
Giant cell arteritis and polymyalgia rheumatica
Takayasu's arteritis
Behçet's disease
Mesenteric inflammatory veno-occlusive disease
Thrombangiitis obliterans (Buerger's disease)

2. Some clues to the pathophysiology in endothelial inflammation

The pathogenetic action mechanisms of aPL are very variable. When binding to membrane phospholipids aPL may inhibit reactions catalyzed by them in the coagulation cascade, for example through inhibition of protein C and S activation. These antibodies may also activate endothelial cell thrombin formation. The binding of aPL with platelet membrane phospholipid-binding protein predisposes to platelet activation and adhesion, with consequent thrombus formation. aPL probably also participate in the complement system activation. As a result, aPL demonstrate proadhesive, proinflammatory, and prothrombotic effects on endothelial cells. The activity of aPL may also be linked to the development of early atherosclerosis in SLE patients.

The activation of monocytes and endothelial cells by aPL can lead to the secretion of a whole variety of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFNγ), etc. The interplay of these cytokines might be one piece in the puzzle surrounding induction of vasculitis in the course of the APS.

Studies using gene array techniques to identify the genetic pattern stimulated by aPL-binding in endothelial cells have revealed induction of a complex proinflammatory, as well as, a proadhesive and procoagulant milieu, which could potentially be involved in the pathogenesis of the APS. It is intriguing that many of the most highly upregulated genes in this study were chemokines, which are involved in recruitment, chemotaxis, and proliferation of mononuclear cells and/or granulocytes (Hamid et al., 2007). These markers of endothelial cell activation are upregulated with prominent deposition of aPL in heart valves, suggesting that aPL deposition initiates an inflammatory process that recruits complement leading to the valve lesion. Autoantibody-mediated endothelial cell activation probably plays a role in sustaining a proadhesive, proinflammatory, and procoagulant phenotype. The heterogeneity of APS clinical manifestations is probably linked to the varied effects that aPL can induce on endothelial and monocytic cells.

Moreover, immune complexes containing oxidized low-density lipoprotein (oxLDL), anti-β_2 glycoprotein I (anti-β_2GPI), and IgG anti-β_2GPI antibodies have also been detected in inflammatory lesions of APS vasculitis. In addition to the induction of inflammatory features in vasculitis, recent data give good evidence that aPL seem to be crucially involved in the developing vasculopathy and atherosclerosis lesions in SLE patients (Nakamura et al., 2006).

3. Cutaneous vasculitis

Several skin lesions have been found in patients with this syndrome, including livedo reticularis, livedoid vasculitis, thrombophlebitis, cutaneous infarctions (Fig. 1), and gangrene of the digits, ulcerations, lesions resembling vasculitis (nodules, macules), cutaneous necrosis/infarctions, subungueal splinter hemorrhages, and, less commonly, discoid lupus and Degos' disease (malignant atrophic papulosis). In 1993, Asherson and Cervera reviewed the main clinical aspects of the dermatologic features of APS (Asherson and Cervera, 1993). Leukocytoclastic vasculitis has been documented in association with the primary APS (Jeffrey et al., 1989) and with SLE (Alarcón-Segovia et al., 1989; Goldberger et al., 1992). It has also been reported in a patient with ankylosing spondylitis and APS (Karter et al., 2002). Stephansson et al. studied skin manifestations in LAC-positive and -negative SLE patients and found that necrotic ulcers appearing at the

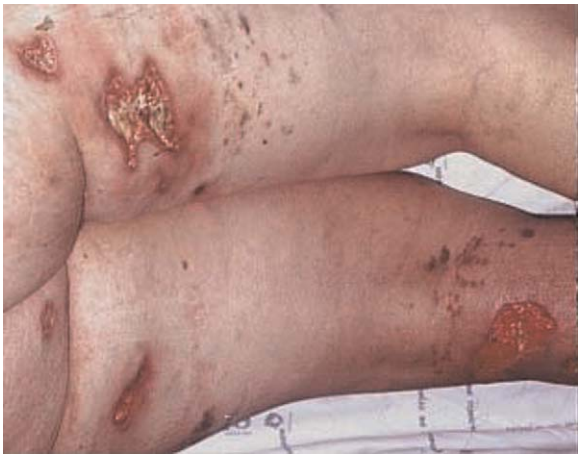

Fig. 1. Extensive skin necrosis in a patient with primary APS who presented with severe pulmonary hypertension and cardiac failure. (See Colour Plate Section.)

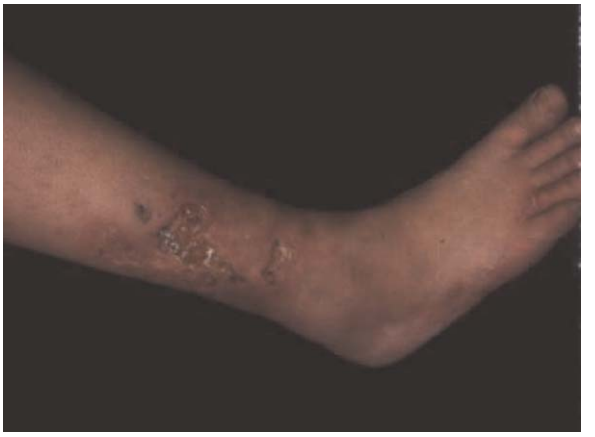

Fig. 2. Lucio's phenomenon in a leprosy patient with raised IgG and IgM anticardiolipin antibodies that were β_2 glycoprotein I-dependent. (See Colour Plate Section.)

beginning of the disease process characterized the 33 LAC-positive patients. Thirteen patients had a "peripheral vascular syndrome"—small leg ulcers of livedoid vasculitis type following deep venous thromboses, which in three patients developed into pyoderma gangrenosum-like ulcers and in two patients into pseudo-sarcoma Kaposi. The lesions were characterized histologically by capillary angiogenesis with extravasated red blood cells, sparse inflammatory cell infiltrates, and microthromboses. Three patients had ulcers clinically and histologically resembling those seen in Degos' disease. Five patients had anetoderma histologically showing elastic tissue depletion and microthromboses. A different pattern of skin changes was seen in the LAC-negative patients. These findings suggest that there is a pathogenetic role of aPL in the described skin manifestations of LAC-positive SLE patients (Stephansson et al., 1991).

Skin nodules and macules resembling vasculitis have been described in two APS patients. The first had a long history of recurrent painful nodules and had also had two deep vein thromboses in the past. The second developed a rash on the lower limbs, resembling vasculitis, which did not respond to prednisolone, but to low-dose salicylate therapy. Histology in both patients revealed microthrombosis of cutaneous vessels (Asherson et al., 1992).

Livedoid vasculitis, otherwise known as segmental hyalinizing vasculitis or livedo reticularis with summer ulceration, is a chronic disease with lesions affecting the feet and lower legs. Early lesions show petechiae, but characteristic features are recurrent, bizarrely shaped ulcers that heal to leave hyperpigmentation and atrophie blanche. The histology shows fibrin deposition within both the wall and the lumen of affected vessels. The absence of a sufficient perivascular infiltrate or leukocytoclasia argues against a vasculitis, being more in keeping with a thrombo-occlusive process. In 1999, Acland and colleagues described four patients with livedoid vasculitis with skin ulceration, all of whom had associated raised aCL but no other evidence of systemic disease (Acland et al., 1999). The term livedoid vasculitis may not be appropriate for this manifestation, because of the scarce or absent vessel wall inflammatory reaction; a more appropriate term would be livedoid vasculopathy, as it has been described associated with APS. It is currently recommended that all patients with this type of lesion should be screened for aPL. The treatment approach should be with anticoagulation, and is obviously different from that for a purely inflammatory condition.

Lucio's phenomenon (Fig. 2) is a rare manifestation of leprosy in which the histopathological findings are related to microvascular thrombosis, without inflammatory cell infiltration in the vessel wall (Fig. 3). A case report has raised the question of the differential diagnosis between Lucio's

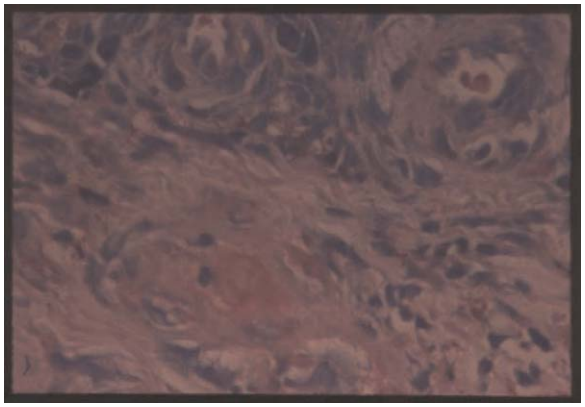

Fig. 3. Histopathology of the skin of the lesion above (Lucio's phenomenon) disclosing microthrombotic lesion without inflammatory infiltration in the cell wall (hematoxylin and eosin, $\times 100$). (See Colour Plate Section.)

phenomenon and APS (Bakos et al., 1996). It is known that leprosy patients may present with raised serum aCL, and in one study of 140 sera from patients with multibacillary leprosy (46 borderline, 94 lepromatous) it was demonstrated that aCL in multibacillary leprosy patients are mainly of the β_2GPI-dependent type with thrombogenic potential (Fiallo et al., 1998). In our experience patients with Lucio's phenomenon had β_2GPI-dependent aCL, with sera characteristics similar to those of APS sera, and different from those of other forms of leprosy, syphilis, or hepatitis C, that are not thrombogenic and do not require β_2GPI for binding to phospholipids (Levy et al., 2000).

Multivariate linear regression analyses in 77 patients with various collagen diseases showed that IgA aCL were independently associated with thrombocytopenia, skin ulcers, chilblain lupus, and vasculitis. Nine patients with IgA aCL alone frequently had vasculitis-associated manifestations, although thrombotic events and recurrent fetal loss were rare. There was also an association between IgM aCL and skin ulcers or chilblain lupus (Tajima et al., 1998).

4. Non-cutaneous organ-specific vasculitis

Three women presenting with acute ischemia of the lower extremities have been described. All were aCL-positive and all required amputation of the affected limb. Histopathological examination was consistent with acute vasculitis of the anterior tibial arteries (Alarcón-Segovia et al., 1989; Goldberger et al., 1992). Ames and colleagues reported vasculitis on the renal artery; this was an unusual case of a 43-year-old man who presented with severe hypertension, oliguria, and seizures (Ames et al., 1992). The erythrocyte sedimentation rate (ESR) was elevated at 60 mm/h and IgG aCL titer was positive. Renal arteriography showed bilateral occlusions and biopsy results showed glomerular ischemia without active vasculitis. Temporal artery biopsy showed IgM, IgE, C3, C4, and C1q deposited in several adventitial areas, suggestive of vasculitis.

In another patient involvement of the retinal vessels and testicular vessels was reported (Rocca et al., 1994) and in a patient with systemic and cerebral vasculitis, disseminated coagulopathy, SLE, and APS was documented by Lie et al. (1995). Weidensaul et al., (1994) also reported a patient with primary APS and vasculitis. At the age of 22 years she had an occlusion of the left posterior cerebral artery with resulting occipital infarction while on oral contraceptives. Seven years later she developed HELLP syndrome and delivery of the stillborn fetus was induced by prostaglandin E_2 suppository. Five days later she developed livedo reticularis, subungual splinter hemorrhages, edema of the lower extremities with elevation of aCL (IgG mainly but also IgM). Renal biopsy revealed segmental necrotizing glomerulonephritis compatible with microscopic polyangiitis. There was no deposition of immunoglobulin or complement. It was thought that the condition had been precipitated by the oral contraceptive initially and then by the puerperium.

5. Systemic vasculitides

5.1. Schönlein-Henoch purpura

Schönlein-Henoch purpura is characterized by palpable non-thrombocytopenic purpura over the lower extremities, arthritis, and abdominal pain with or without gastrointestinal hemorrhage and, less commonly, glomerulonephritis. Histologically,

the disease is characterized by leukocytoclastic vasculitis and is regarded as a specific clinicopathologic entity based on the vascular deposition of IgA-dominant immune complexes. Schönlein-Henoch purpura in adulthood differs from the pediatric form of the disease in that adults have more pronounced skin lesions, and renal involvement is more frequent than in childhood. In the most relevant study on this topic 22 (73%) of 30 Japanese adult Schönlein-Henoch purpura patients were positive for serum levels of IgA aCL and of IgA phosphatidylserine-dependent antiprothrombin (aPS/PT) antibodies. The titers of these antibodies were significantly higher than those in controls. Comparable results have been obtained in a study with children with Schönlein-Henoch purpura (Kawakami et al., 2008). A few case reports describe patients displaying a close relation between Schönlein-Henoch purpura, a history of recurrent thromboses, and the presence of aCL, as recently published by Garber et al. (1993).

5.2. Polyarteritis nodosa

Several case reports documenting an association between polyarteritis nodosa (PAN) and aPL have been published. Praderio et al. (1990) reported a 63-year-old woman with LAC who developed digital necrosis of her left foot. Pathology of the two amputated digits revealed destruction of the vessel walls of the majority of the small and medium sized arteries by a dense polymorphonuclear infiltrate. This patient also had several microinfarcts on brain MRI as well as abnormal lung perfusion scans. Two patients with aPL and PAN were then reported by Norden et al. (1995). The first, a 42-year-old man, presented with abdominal pain and fever. He had had a past history of a femoropopliteal bypass at the age of 32 years. A skin rash showed changes consistent with thrombi and not vasculitis. Mesenteric angiography showed multiple aneurysms of the hepatic artery. An extremely high ESR (130 mm/h), positive antinuclear antibodies (ANA) at 1:320, and elevated IgG as well as IgM aCL were also detected. He was treated with intravenous steroids followed by oral steroids and anticoagulated with heparin and warfarin and recovered well. The second case, a 39-year-old man, presented with fever, myalgia, night sweats, and testicular pain and swelling. Severe weight loss was noted. Mononeuritis multiplex developed and muscle and sural nerve biopsies revealed necrotizing vasculitis, bilateral occipital infarcts accompanied by blindness occurred on prednisone therapy, and positive aCL levels were then detected as well as thrombocytopenia. The ANA was positive at 1:320. Both these patients appeared to have had APS associated with PAN. There was no further evidence of SLE either clinically or serologically.

De La Fuente Fernandez and Gil (1994) described a 58-year-old woman with high levels of aCL, livedo reticularis, and central nervous system involvement (lacunar infarctions) in addition to changes of necrotizing vasculitis and fibrinoid necrosis in skin and muscle biopsy histopathology. A high ESR, platelet count, and C-reactive protein (CRP) were present. Cohney et al. (1995) described an ANCA-positive patient who had circulating LAC. Pereira et al. (1995) documented a child with a diagnosis of juvenile cutaneous PAN with attacks of fever, cutaneous rashes, and gangrene, which resulted in amputations of the distal portions of the fingers and toes; p-ANCA was positive as was aCL determination. Biopsies did not reveal any evidence of vasculitis. Schoonjans et al. (1996) reported a case of biopsy-proven PAN and APS. Hansen et al. (1999) reviewed all patients with either Wegener's granulomatosis or PAN discharged between the years 1993 and 1998 at Duke University. They found four patients with positive aCL determinations. There was no statistically significant association between the presence of these antibodies and aPL-associated clinical events. In general the aCL were present in low titer and repeated testing showed resolution of the antibody. They suggested that the aCL may occur as part of the hypergammaglobulinemia seen in vasculitis or as a result of non-specific binding in the assay for aCL.

Musuruana and Cavallasca reported a male patient with a diagnosis of PAN who, after 7 years of being diagnosed with vasculitis, showed ischemic lesions in his legs associated with high titers of aCL, along with angiographic and histological evidence of thrombosis. Despite immunosuppressive

and anticoagulant therapy, his lesions progressed, and both legs had to be amputated (Musuruana and Cavallasca, 2008).

In a recent study reporting 16 patients with cutaneous PAN features, anti-PS/PT antibodies and/or LAC were detected in all patients, but not in 23 healthy controls nor in 41 diseased controls. Three of the patients with cutaneous PAN were positive for IgG aCL antibody and two for IgM aCL antibodies. In this study the authors proposed that aPL could be used as a new relevant diagnostic factor in the identification of patients with symptoms of cutaneous PAN (Kawakami et al., 2007).

5.3. Wegener's granulomatosis

The finding of aPL in patients with Wegener's granulomatosis is even less frequent than that in patients with PAN. In the Hansen review mentioned above (Hansen et al., 1999), the authors found seven out of 36 patients who were positive for aPL. Two of these had sustained an aPL-associated clinical event. The first, a 46-year-old female had developed transient ischemic attacks after commencing hormone replacement therapy, while the second had sustained a pulmonary embolus at the age of 29, 12 years prior to the onset of the Wegener's granulomatosis. Six years after the onset of the vasculitis he suffered a myocardial infarction. However, five of 33 patients who were aPL-negative suffered thrombotic events. Castellino et al. (2000) reported a case of Wegener's granulomatosis with diffuse pulmonary hemorrhage and aCL as well as a pulmonary embolus. Lamprecht et al. (2000) also recently confirmed the study of Hansen and found that the prevalence of aCL and antibodies to β_2GPI in patients with Wegener's granulomatosis and previous thrombosis did not differ from that in patients with Wegener's granulomatosis without previous thrombosis.

Relapsing splenic vein thrombosis, a very rare complication of Wegener's granulomatosis, has been described in a female patient. The positive aPL found in this case are a rare occurrence in primary vasculitis, especially in Wegener's granulomatosis. It probably caused or accentuated an effect of the Wegener's granulomatosis on the splenic vein.

A recent study analyzing the association between aPL-induced thrombocytopenia in patients with Wegener's granulomatosis showed that it only had a minor effect in this disease (Meyer et al., 2001).

5.4. Relapsing polychondritis

Venous thrombosis has been observed in over 10% of patients with relapsing polychondritis (Vinceneux et al., 2000). Patients may either have superficial thrombophlebitis or deep venous thromboses, which may be complicated by pulmonary thromboembolism and may be recurrent. Retinal vein (Isaak et al., 1986) and superior vena cava obstruction have been documented in a patient who had a large aneurysm of the ascending aorta (Sohi et al., 1981). Although the pathophysiology of the venous involvement is multifactorial, aPL have been implicated in a few case reports (Pouchot et al., 1996; Piette et al., 1997; Empson et al., 1998). In a multicenter study of 21 patients with relapsing polychondritis, aCL were found to be elevated in eight patients. No patients had elevated anti-β_2GPI and none had clinical signs and symptoms of APS. The two patients with the highest aPL had concomitant SLE, suggesting that elevated aPL in relapsing polychondritis is more closely related to SLE than to the relapsing polychondritis itself. There is no convincing evidence that aPL are associated with relapsing polychondritis (Zeuner et al., 1998). In recent years very few case reports showing aPL in combination with polychondritis have been found (Roux et al., 2004).

5.5. Capillaritis

Microvascular injury manifesting as a "capillaritis" is an entity that was defined by Lie (1996). This may coexist with aPL as has been described in several cases (Gertner and Lie, 1993; Crausman et al., 1995). The capillaries are single-cell vascular loops without external vascular smooth muscle cells. The diagnosis of "capillaritis" can only be inferred by the "spilling" of inflammatory cell infiltrate with granulocyte karyorrhexis as well as erythrocyte extravasations over the affected capillary network

and the capillary loops may be damaged beyond recognition. This results in acute necrotizing glomerulonephritis in the kidney and pulmonary capillaritis when the lung is involved. Often alveolar hemorrhage or microthrombosis may coexist with the capillaritis (Asherson and Greenblatt, 2001).

In a recent study with four cases, patients presented with dyspnea, hemoptysis, fever, hypoxia, and diffuse alveolar infiltrates; none had evidence of acute thromboembolic disease. All secondary causes of diffuse alveolar hemorrhage (DAH) were ruled out. All patients tested positive for the LAC and high-titer aCL, including anti-β_2GPI. Three cases had lung biopsies that revealed pulmonary capillaritis and DAH with no evidence of thrombosis. The cause of this disease was thought to be aPL-mediated endothelial cell activation in the absence of thrombosis that might have induced the capillaritis observed (Deane and West, 2005).

5.6. *Superficial thrombophlebitis*

Although mentioned in early reports and reviews on the APS, recurrent superficial thrombophlebitis is an uncommon occurrence in the APS compared with deep venous occlusions. When de Godoy et al. (2001), however, compared patients with this condition with 100 voluntary donors from the blood bank, they found that 33.3% (15 patients) with thrombophlebitis were positive for aCL, the majority demonstrating the IgM isotype, and in the control group aCL was found in 7%. These results suggest a positive correlation between aCL and recurrent superficial thrombophlebitis. In addition, only a few case reports refer to this rare feature of the APS (Boehlen et al., 2004).

6. Giant cell arteritis and polymyalgia rheumatica

Arterial occlusions are serious complications that may occur in giant cell arteritis (GCA) but not in polymyalgia rheumatica (PMR). A report by Cid and colleagues of a 76-year-old female with GCA with high IgM aCL who developed recurrent arterial thrombosis, raised the question whether aCL could trigger thrombosis in these patients, although severe atherosclerosis was observed in the patients' autopsy (Cid et al., 1988). The same group of authors studied 40 patients with GCA, 13 of whom had previous ischemic events. Three had aCL, all of whom were within the group with previous ischemic events, and two had thrombosis while "in remission" from the vasculitic syndrome with no other features of APS (Cid et al., 1990).

IgG aCL were found in 11 of 22 patients with GCA studied by McHugh and colleagues. They noted that aCL were more frequently related to acute disease and concomitant PMR and that the antibody levels decreased with corticosteroid treatment (McHugh et al., 1990). A study from Florida with 50 patients, 30 with PMR alone and 20 with associated GCA, found aCL in 48%. Eleven were positive for IgG and 5 for IgM; 8 were positive for both. In the group of patients with PMR alone, aCL was detected in 26.6%, whereas 80% of those with GCA had raised aCL (Espinoza et al., 1991). Another study with 29 northern Italian patients with biopsy-proven GCA and PMR, with early disease, prior to corticosteroid use, found no correlation between the presence of aCL or LAC and any of the variables studied (Salvarani et al., 1992).

Meyer et al. (1996) studied 19 patients with GCA, including 16 with concomitant PMR and three with isolated PMR. IgG aCL were found in 8 patients (36%) and IgG anti-β_2GPI antibodies in 2 (9%), including one without aCL. Of the 8 patients with aCL, 2 had GCA alone and 6 had PMR with clinical ($n = 2$) or histologic ($n = 4$) evidence of GCA. All patients tested negative for LAC. Positivity for aCL was significantly related to elevated ESR, and higher serum CRP and fibrinogen values. Patients with aCL had a tendency to have a higher platelet count and to be on a lower prednisone dosage. The latter may account for the correlation between aCL and laboratory markers for inflammation.

These data, including serial measurements, suggest that aCL are present early in the course of GCA and disappear after weeks of beginning corticosteroid therapy. The authors concluded that positive aCL or anti-β_2GPI titers in patients with PMR suggest a diagnosis of concomitant GCA, which is usually symptomatic (Meyer et al., 1996).

Seriolo et al. (1998) studied 28 patients with PMR, 20 with associated GCA and found a positive correlation with elevated lipoprotein(a) that was significantly higher in those with aCL and thrombosis as opposed to those with aCL without thrombosis. This correlation was even more prominent in the patients with aCL and arterial thrombosis (Seriolo et al., 1998). An elegant multicenter, prospective case–control study was performed in order to assess the prevalence and thrombogenic role of aCL in 284 patients with GCA or PMR, whose samples were obtained at the time of diagnosis, and 210 randomly selected age- and sex-matched controls. Positive aCL titers were present in 20.7% of patients and 2.9% of controls. There was a positive relationship between high aCL and findings of GCA on biopsy ($p = 0.04$). Although aCL were associated with thrombotic complications in univariate analysis, the biopsy findings remained the only predictive variable in stratified analysis. The authors concluded that in GCA, aCL seem to function as reactive antibodies in relation to endothelial lesions (Duhaut et al., 1998).

IgG and IgM anti-β_2GPI antibodies were not detected in any of the 45 unselected patients with biopsy-proven GCA, including 15 patients with ischemic events, studied in 1998 by Liozon and colleagues, while 51% had elevated aCL, but without correlation with ischemia (Liozon et al., 1998). In a retrospective fashion, Hulin et al. (1999) studied aPL prevalence and its relationship with ischemic events in 62 patients with GCA and/or PMR. Before corticosteroid treatment, 41% of the 51 patients with GCA and 64% of the 11 patients with isolated PMR had high IgG aCL levels, a frequency significantly higher than that in the control group, composed of healthy elderly people. The authors found no correlation between the occurrence of an ischemic event and the presence of aCL. As in other studies, aCL disappeared soon after corticosteroid therapy was initiated.

Clinical and laboratory evidence of APS was found in only three of 248 with GCA and/or PMR followed at the University Hospital of Padova in Italy (Ruffatti et al., 2000). In a longitudinal study lasting 24 ± 11 months, Liozon and colleagues measured aCL and CRP levels in 58 patients with biopsy-proven GCA in order to evaluate whether elevated aCL is a predictor of relapse. Before treatment, aCL were found in 27 cases (46.6%) and levels decreased with appropriate treatment in all patients except one, after a variable delay. The aCL levels did not increase in any patient whose disease was permanently controlled. A significant rise in aCL was recorded in 20 of 27 (74%) of the flares or relapses of GCA. The authors concluded that aCL levels are useful in the detection of flares and relapses in GCA with fairly good sensitivity (74%) and a specificity of 100%, and that it can be used to distinguish subclinical flares from infection (Liozon et al., 2000).

A recent study included 80 patients with established GCA: 36 had isolated GCA, 14 had isolated PMR, and 30 had GCA and PMR. Forty-four patients (67%) had ischemic phenomena due to GCA. A control group of 100 age- and sex-matched individuals was also analyzed. All participants were tested for the aPL profile, as well as for protein C, protein S, antithrombin activity, factor V Leiden mutation, and prothrombin gene G20210A mutation. Fibrinolysis parameters, such as plasminogen, tissue-type plasminogen activator (t-PA) antigen, t-PA activity, type 1 plasminogen activator inhibitor (PAI-1) antigen, PAI-1 activity, and the 4G/5G polymorphism of the promoter region of the PAI-1 gene were also studied. The authors found 11 patients (18%) that tested positive for LAC, 24 (30%) for aCL, 9 (11%) for anti-β_2GPI, and 29 (36%) for antiprothrombin antibodies. No relationship was found between these autoantibodies and ischemic manifestations. None of the patients had decreased physiological coagulation inhibitors. Two patients and two controls were heterozygous for factor V Leiden, and only one patient and two controls were heterozygous for the prothrombin gene G20210A mutation. No statistically significant correlation was found between any thrombophilic factor and GCA-related or GCA-unrelated ischemic events. The authors concluded that their GCA patients have a high prevalence of aPL that is not related to ischemic manifestations (Espinosa et al., 2000).

In spite of the large number of publications there is still no consensus whether the presence of aCL in GCA is an important risk factor for the development of thrombotic complications. In addition, it is noting that after this last extensive study no

additional efforts have been made to clarify the pathophysiological impact of aCL in GCA.

7. Takayasu's arteritis

Like the other reports of aPL in most of the primary necrotizing vasculitides, the ones related to Takayasu's arteritis are mainly case reports or small series of patients. It is important to remember that the presence of aPL may not be accompanied by the clinical features related to APS. In the past the thrombotic nature of Takayasu's arteritis has been related to elevated levels of B-thromboglobulin, platelet factor 4, thrombin–antithrombin III complex, and fibrinopeptide A. Shin and Godwin (1999) reported a case of Takayasu's arteritis associated with activated protein C resistance related to factor V Leiden mutation. Girona et al. (1993) reviewed the immunological profile of 21 patients with untreated Takayasu's arteritis. They found that the occasional aPL that may be found in these patients are not related to the characteristic clinical features of APS nor do they have any other clinical relevance. This study suggests that there is no support for a significant role of humoral immunity, coagulation, and fibrinolysis in the pathogenesis of Takayasu's arteritis.

Yokoi et al. (1996) reported two patients with Takayasu's arteritis in whom LAC was found and the aCL was elevated. The first developed diffuse stenosis and occlusive changes in larger arteries. The second had small-sized arteries showing occlusive vasculitis without thrombosis on histology, in addition to findings in the large-sized arteries compatible with Takayasu's disease. The authors state that these findings, which are uncommon in Takayasu's arteritis, suggest that aPL may have contributed to the pathogenesis of the extensive vasculopathy and may have triggered the vasculitis in these patients. These findings are somewhat controversial.

Misra et al. (1994) screened 34 patients fulfilling the criteria for Takayasu's arteritis, and 50 normal controls for ANA, rheumatoid factor, and aCL. They found moderate or high IgG aCL in 14 patients (41%), mostly in the group with active disease. Controversially, the authors questioned the role of aPL in the pathogenesis of the vasculopathy found in Takayasu's arteritis.

In a recent report 47 patients with Takayasu's arteritis and 30 age- and sex-matched controls were studied. Besides the high percentage of antibodies directed to endothelial cells ($>80\%$) no significant detection of aCL antibodies (in 4 patients) could be revealed (Park et al., 2006). The authors of this paper state that the detection of aCL in patients with the Takayasu's arteritis is not of major diagnostic importance.

8. Behçet's disease

There is a wide variation in the frequency of aPL reported in Behçet's disease, from 0 to 50%. Few studies have demonstrated a correlation with specific manifestations: Hull et al. (1984) reported 19% positivity of aCL and found a relationship between IgM aCL and retinal vasculitis. Pereira et al. (1989) observed IgG aCL in 3 of 10 patients with Behçet's disease and ocular disease, and a lower frequency in those taking steroids. An association of IgM aCL with cutaneous vasculitis and erythema nodosum was reported by Zouboulis et al. (1993). Karmochkine et al. (1993) studied 19 patients with Behçet's disease, 11 of whom had previous thrombosis; they found no correlation with any type of aPL in this population.

A recent study by Tokay et al. (2001) included the largest number of patients reported. They found the frequency of aCL to be lower in Behçet's disease (2.4%) than in SLE (50%) or normal controls (5.6%). The authors were unable to find any significant clinical association.

Genetic differences and technical disparities in the assays employed may account for the discrepancies in the findings. There are several clinical and laboratory manifestations of Behçet's disease, such as gastrointestinal involvement and positive pathergy reaction, that present with different frequencies depending on the ethnic and geographic background of the studied population. The same seems to be the case for aPL presence and APS diagnosis.

9. Mesenteric inflammatory veno-occlusive disease

This recently described variety of vasculitis, which results in small bowel infarction, has also been associated with aCL (Gul et al., 1996). The association between venous mesenteric occlusion and peripheral venous thrombosis has been recognized for a long time (North and Wollenman, 1952). It has also been known that early recurrent thrombosis at the site of anastomosis following bowel surgery is quite high, especially in those with a tendency to spontaneous venous thrombosis (Jona et al., 1974).

10. Buerger's disease

Superficial thrombophlebitis is an integral clinical presentation of Buerger's disease (thromboangiitis obliterans). This condition is a segmental occlusive inflammatory disorder of arteries and veins most commonly affecting the lower extremities of young male cigarette smokers and its etiopathogenesis is still obscure. It may involve vessels of the upper extremities, mesenteric or coronary vessels. Its relationship to thrombogenic risk factors has been studied by several investigators. Heterozygosity for the recently described prothrombin gene 20210 A and factor V Arg506 to Gln (factor V Leiden) has been described on one patient (Mercie et al., 1998), protein S deficiency in another (Athanassiou et al., 1995), hyperhomocysteinemia in three patients (Caramaschi et al., 2000), and high levels of lipoprotein (a) in another (Biasi et al., 1999). All these are risk factors for thrombosis. There are isolated reports of aCL being detected in some of these patients (Jimenez-Paredes et al., 1998), as well as anti-endothelial cell antibodies (Eichhorn et al., 1998; Adar et al., 2000).

There is still no consensus about diagnostic criteria, and several authors argue that the occurrence of a detectable coagulation disorder, systemic disease, or embolism would rule out the diagnosis of Buerger's disease (Adar et al., 2000). Some authors have associated Buerger's disease with APS, as well as hyperhomocysteinemia. Other thrombophilic conditions, as mentioned, have been described anecdotally in patients with Buerger's disease. A recent study of 36 patients with Buerger's disease investigated the prevalence of prothrombin 20210 G→A, factor V 1691 G→A (factor V Leiden), and factor V 4070 A→G (His1299Arg) mutations. The prothrombin gene mutation was found to be the only one associated with increased risk of vascular thrombosis in these patients ($p = 0.032$) (Avcu et al., 2000).

In view of the developing line of investigation, there is a clear need to redefine the diagnostic algorithm and the criteria for diagnosing Buerger's disease (Olin, 2000). In one smaller study in patients with Buerger's disease the prevalence of aCL was significantly higher in patients with thrombangiitis obliterans (36%) than in either patients with premature artherosclerosis (8%) or healthy individuals (2%). Patients with thrombangiitis obliterans and a high antibody titer tended to be younger and to have a significantly higher rate of major amputations than those without the antibody (100% vs. 17%). Clinical features of thrombangiitis obliterans not significantly altered by the presence of aCL included upper limb involvement, digital necrosis, and superficial thrombophlebitis (or deep venous thrombosis) (Maslowski et al., 2002).

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CHAPTER 14

Antiphospholipid Antibodies and Atherosclerosis

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1. Introduction

The antiphospholipid syndrome (APS) is a complex disorder of coagulation linked to a characteristic set of autoantibodies. The syndrome has been reclassified several times to construct clinical criteria that can facilitate research (Wilson et al., 1999; Lockshin et al., 2000; Wilson et al., 2001; Miyakis et al., 2006). However, APS can be found not only as a primary disease but also in the context of numerous other autoimmune, infectious, or neoplastic diseases (Grossman, 2004; Marai et al., 2004; Pugliese et al., 2006; Ostrowski and Robinson, 2008), the most striking of which is its very strong association with systemic lupus. It is also clear that a range of vascular abnormalities can arise involving autoantibodies and clinical manifestations which overlap with or are closely related to APS, suggesting that a spectrum of disease exists in these patients beyond the strictly defined APS classification critera (Nojima et al., 2001; Sanmarco et al., 2001; Reichlin et al., 2002; Bertolaccini et al., 2003; Arai et al., 2003; de Carvalho et al., 2004; Zanon et al., 2004; Amoroso et al., 2003; Carmo-Pereira et al., 2003; Forastiero et al., 2003; Lopez et al., 2004; Iverson et al., 2006; Batuca et al., 2007).

This has led to controversy about the classification criteria (Swadźba et al., 2007; Tarr et al., 2007; Baker et al., 2008; Carvalho, 2008; Galli et al., 2008; Tripodi, 2008), an ongoing discussion which merits acknowledgment before considering whether or how antiphospholipid antibodies (aPL) might contribute to risk for atherosclerosis. The importance of maintaining a strict classification scheme for APS cannot be underestimated in order to facilitate comparative studies and advance safer and more effective treatments for this life-threatening disorder. However, since a wider array of autoantibodies and clinically meaningful vascular abnormalities have been identified (and in most cases corroborated) within the same patient population, it would not be clinically meaningful to ignore many of the most likely culprits in potential associations between APS and atherosclerosis. The definition of aPL, then, for the purposes of this review, will include any of the currently identified autoantibodies to phospholipids and/or intravascular, phospholipid-binding proteins (including proteins embedded in membranes of cells or proteoliposomes) that have been associated with APS (Fig. 1). An emerging theme in autoimmunity which will also be explored in this review is that not all autoantibodies must necessarily be detrimental to a host; some which target vascular regulators might even be protective against disease.

It is important to keep in mind that atherosclerosis itself is multifactorial and the study of this condition contributes its own set of imponderable variables (Blake and Ridker, 2001). Therefore, in sorting through the disparate reports that either support or refute an association between aPL and atherosclerosis, an important caveat is that most of these studies are underpowered for the multiple likely variables impacting on outcomes, and that the modeling of these variables is as different from

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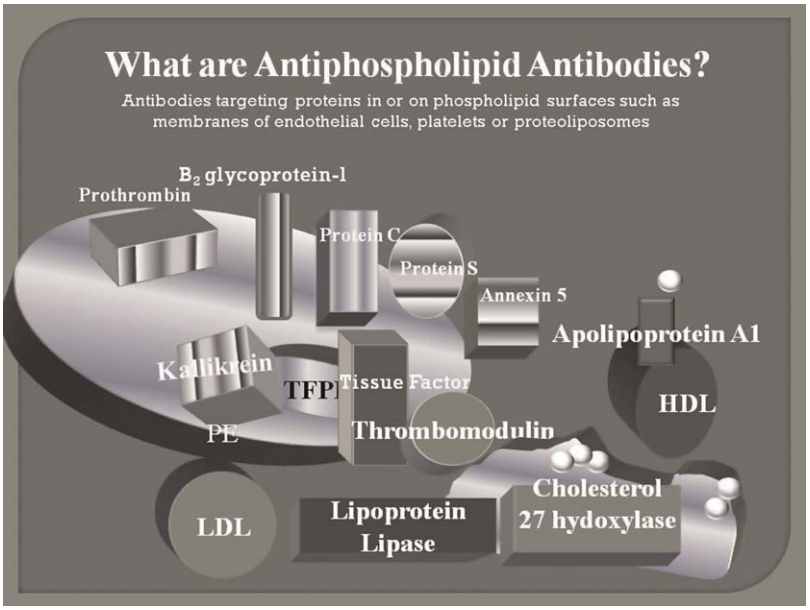

Figure 1. Antiphospholipid antibodies.

report to report as is their background prevalence in each of the populations studied. In light of this problem, the suggestion that different autoantibodies might play specific regulatory or disregulatory roles in human lipid metabolism, plaque evolution or critical end-stage thrombotic events remains as intriguing as ever, but cannot be definitively proven or disproven at the time of this review. Nevertheless, the weight of the literature suggests that this is the case.

2. Clinical evidence for or against a relationship between antiphospholipid antibodies and atherosclerosis

The risk for premature atherosclerosis in autoimmune patients such as those with lupus is known to include traditional risk factors, chronic generalized inflammation, and target-specific autoimmune mechanisms (Doria et al., 2005; Zampieri et al., 2005). The effects of all of these elements are likely exacerbated by chronic use of medications such as corticosteroids which add an additional disease burdon by worsening obesity, hyperglycemia, and hyperlipidemia. Since both traditional and autoimmune mechanisms are known to contribute broadly to atherosclerotic plaque over time (Doria et al., 2005), and since, as will be reviewed below, aPL and related autoantibodies may in turn induce some of the more traditional risk factors, it is probably not possible to sort out an independent effect of aPL on clinical cardiovascular endpoints, although some interesting data from broadly modeled studies are available.

Whether or not APS contributes to premature atherosclerosis, the APS population represents a unique subset of people, being younger, more likely to be female, and less likely to smoke than other patients with arterial disease (Shortell et al., 1992). In addition, it has been suggested that antibodies to β_2 glycoprotein I (β_2GPI) may be a significant risk factor for myocardial infarction in young premenopausal women, independently of other risk factors such as smoking, hypertension, and the degree of coronary artery stenosis (Meroni et al., 2007).

In a study of 200 women with systemic lupus erythematosus (SLE) and 100 controls, SLE patients who were less than 55 years old had more plaque overall and more evidence of plaque in the internal carotid artery (Ahmad et al., 2007).

Variables relevant to risk in these SLE patients included age, smoking, previous arterial events, exposure to immune suppression (potentially a surrogate for more severe inflammatory disease), and aPL (Ahmad et al., 2007). The complexity of vascular complications in patients with SLE is illustrated by a 1996 report from the prospective cohort of lupus patients at Johns Hopkins. Several apparently independent factors seemed to influence risk for thrombosis, including evidence of immune complex-mediated inflammation (high anti-dsDNA and low C3), ubiquitous risk factors for atherosclerosis (hypertension, hyperlipidemia, and homocysteine) and, as an apparently independent factor, the presence of aPL (Petri, 1996). In another study of 155 patients with SLE using a logistic regression model that included several autoantibodies and traditional risk factors antibodies to cardiolipin and β_2GPI comprised the most significant risk factor for atherosclerosis (Nojima et al., 2008).

Another report examined evidence for atherosclerosis in 28 patients with primary APS and 28 age- and sex-matched controls, each of whom underwent high-resolution B mode carotid ultrasonography and spectral analysis (Medina et al., 2003). APS patients had more frequent increased carotid artery intimal media thickness (IMT) and decreased lumen diameter than controls, which was not associated with classical cardiovascular risk factors such as hyperlipidemia, diabetes, smoking, obesity, or hypertension (Medina et al., 2003). Furthermore, those with increased IMT had a threefold higher risk for stroke than those without, suggesting the possibility that atherosclerosis may be a feature of APS-attributed stroke.

Thirty-three premenopausal women with APS were compared with patients with SLE, patients with rheumatoid arthritis, and healthy controls. Women with both primary APS and SLE seemed to have more advanced carotid and femoral plaque that was not accounted for by other risk factors for atherosclerosis, including age, hyperlipidemia, or cumulative steroid use (Vlachoyiannopoulos et al., 2003). In another study, carotid IMT was evaluated in 29 patients with primary APS and compared with that in 13 with persistence of antibodies without evidence of a thrombotic disorder (Ames et al., 2002). IMT was greater in thrombotic than non-thrombotic subjects and this correlated with age and inflammatory markers. In a regression analysis, the titer of anticardiolipin antibodies (aCL) independently predicted IMT at multiple areas of the carotid arteries (Ames et al., 2002). A smaller study comparing carotid abnormalities in patients with primary APS vs. controls confirmed this increase, but only in patients who over the age of 40 (Ames et al., 2005). In a study including 58 APS patients and 58 controls a significant difference was found between IMT and arterial stiffness when comparing patients and controls (Belizna et al., 2008a) which were independent of other cardiovascular risk factors. No differences in plaque were found between patients with primary APS and those with secondary APS (Belizna et al., 2008a).

Mean flow-mediated brachial dilatation (FMD), which is a surrogate measure for endothelial dysfunction, has been reported to be significantly lower in patients with primary APS than in age- and sex-matched controls, accompanied by various serum measures confirming that endothelial function is impaired (Stalc et al., 2006). FMD of the brachial artery has also been reported to be lower in patients with the primary APS (and those specifically with aCL) than in controls even when IMT was similar (Bilora and Sartori, 2008). Anticardiolipin antibodies were also associated with lower FMD in a study of 107 patients who had been referred to a vascular laboratory (Marai et al., 2008).

Of 546 patients in the prospective, multicenter LUMINA study, predictors for subsequent cardiovascular events included traditional risk factors such as smoking, but also both aPL and CRP supporting roles for both inflammation and autoimmunity in the development of accelerated atherosclerosis in SLE (Toloza et al., 2004).

There were clearly many differences in the reports listed above, in the populations studied, in the variables measured and in the type of analysis performed but aPL were repeatedly implicated in these models. Not all studies have reached the same conclusion, however. In one comparison of 85 primary APS patients and 40 controls with deep venous thrombosis from a different cause no increased risk for evidence of atherosclerosis was found in

the APS patients (Bilora et al., 2002). Another very carefully performed cross-sectional study of SLE patients at Cornell University failed to demonstrate an association of aPL and carotid atherosclerosis (Farzaneh-Far et al., 2006). This same group has correlated atherosclerosis progression in a prospective study to homocysteine concentration, and less aggressive immune suppression (Roman et al., 2007), suggesting that multiple factors, especially inflammatory variables, could be confounding the impact of aPL in a lupus population. Interestingly, high levels of homocysteine have been correlated with the presence of lupus anticoagulant (LAC) in patients with cerebral ischemia (Chen et al., 2007), suggesting the possibility that in some populations the impact of aPL could be indirect and thus difficult to detect as an independent effect from other variables they may be affecting.

In an earlier case–control study by the Cornell group of 197 patients with lupus and 197 matched controls, carotid plaque was, as expected, found more than twice as often in SLE patients than in controls (Roman et al., 2003). Less immune suppression and age were the major risk factors for plaque in this population, and there were fewer of the classic aPL detected in the group with atherosclerosis, however this might be expected in lupus patients as they get older. The major contribution of the negative studies might be the extent to which lupus disease activity may be contributing to risk for atherosclerosis. Treatment history appears to have an impact in a number of the cohorts, and could be a reasonable surrogate measure for vascular inflammation over time. However, since not all studies have agreed on the effect of more or less immune suppressive treatment, it could be that in some populations, aggressive treatment denotes appropriate control of vascular inflammation but in others it reflects a more severely diseased population, which may not have been controlled by the frequency or level of treatments given. Steroids compound the analysis problem, since they may decrease vascular inflammation in some important ways while increasing arterial disease in other ways. Thus treatment differences per se are hard to interpret.

In a report published in the Hopkins Lupus Cohort in 2004, myocardial infarction was found to occur significantly more often in those with LAC, however there was no association between aPL and a cross-sectional evaluation of carotid IMT, carotid plaque, or coronary calcium by helical computed tomography (CT) (Petri, 2004). This might denote an association between the LAC and thrombotic but not atherosclerotic risk, or it might simply illustrate a problem with cross-sectional studies, since a cross-sectional evaluation of potentially fluctuating autoantibody titers does not necessarily reflect cumulative damage that may have led, over many months or many years to abnormalities in carotid IMT or end-stage cardiovascular events.

In summary, a number of studies suggest that premature atherosclerosis might be associated with the APS, however not all clinical-based papers agree (Table 1). None of these studies were designed to prospectively measure all of their cardiovascular endpoints repeatedly, and by consistent techniques, over many years, and all would be considered underpowered to analyze the multiple variables that

Table 1
Antigens targeted by antiphospholipid syndrome-related autoantibodies

Antigens[a]	References
Coagulation-regulating proteins	
Protein C	Nojima (2001)
Protein S	Nojima (2001) and Bertolaccini (2003)
Annexin 5	Nojima (2001) and Arai (2003)
Prothrombin	Nojima (2001) and Zanon (2004)
β_2 Glycoprotein I	Nojima (2001) and Amoroso (2003)
Tissue factor plasma inhibitor	Forastiero (2003)
Physiologic phospholipids	
Phosphatidylserine	Lopez (2004)
Phosphatidylethanolamine	Sanmarco (2001)
Antigens for IgA antibodies	
β_2 Glycoprotein I	Iverson (2006)
Cardiolipin	Carmo-Pereira (2003)
Regulators of lipid metabolism	
Lipoprotein lipase	Reichlin (2002) and de Carvalho (2004)
HDL (with associated paroxynase decrease)	Batuca (2007)
Apolipoprotein A1	Swadźba (2007)
Oxidized LDL	Ames (2002)

[a] Partial list to demonstrate examples.

have been suggested to impact on cardiovascular risks in patients with autoimmunity. A more definitive verdict awaits a more comprehensive, prospective study, preferably with an inception cohort of early lupus patients, which can collect a rich database of information prospectively about major and minor cardiovascular risk factors, medication changes, cardiovascular events, and the broad range of aPL and related autoantibodies discussed below. Such a study would need to be powered to better analyze the multiple potential confounding risk factors.

Such a study is underway. The Systemic Lupus International Collaborating Clinics registry for atherosclerosis includes 27 centers from 11 countries and has assembled a registry of over a thousand patients diagnosed with SLE within 18 months of study entry, with the goal of a 10-year follow-up for 1500 early lupus patients. In a preliminary report it is evident that classical risk factors for atherosclerosis increase over the first 3 years of enrollment in this population (Urowitz et al., 2008). The impact (or lack thereof) of aPL may be more apparent when this prospective cohort matures.

3. Classic antiphospholipid antibodies: can they explain all autoimmune mechanisms for atherosclereosis or are they the tip of an iceberg?

The spectrum of autoantibody profiles for primary APS and that of APS secondary to SLE are known to be similar (Soltész et al., 2003). Subtypes of classic aPL (such as LAC, aCL, or anti-β_2GPI) have been studied for many years with variable results relating each to different types of vascular disease (Carmo-Pereira et al., 2003; Soltész et al., 2003; Galli, 2004; Lopez et al., 2004; Palomo et al., 2004; Galli and Barbui, 2005; Nojima et al., 2006). In one recent report, venous thrombosis was found to occur more frequently in people with LAC, while some arterial disease was associated with aCL but cerebrovascular thrombosis seemed to correlate with either LAC or IgG aCL (Soltész et al., 2003). However, association of different types of APS-associated autoantibodies with atherosclerosis endpoints remains controversial (Galli, 2004), and the integration of this knowledge may be hampered by the different populations that have been studied in various reports and lack of standardization of laboratory tests around the world. Few studies have integrated more than two or three known autoantibodies into multivariate models, and different studies have incorporated different subsets of these antibodies, further complicating the interpretation of these tests.

In this context, how wide a net should be cast for aLP when considering their contribution to atherosclerosis? There is no community consensus on this problem, and there is certainly no definitive data in support of one model for APS-induced thrombosis vs. another. Without doubt, there are overlaps between the autoantibody profiles of patients with SLE and no thrombotic history, APS secondary to SLE, and patients designated as having primary APS in the absence of other autoimmune features. It could logically, then, be considered that these syndromes exist along one continuum of pathology. In support of this speculation, one study has suggested that the presence of antibodies that target oxidized low-density lipoprotein (oxLDL) is associated with a history of arterial thromboses in patients with both primary and secondary APS and is also associated with the presence of anti-β_2GPI antibodies (Bećarevic et al., 2005), which is common in SLE and represents one of the diagnostic antibodies accepted for the APS (Bećarevic et al., 2005). This suggests a relationship of APS autoantibodies to a lipid-regulating structure with relevance both to atherosclerosis and to the APS, in the presence and absence of SLE.

Pengo and colleagues evaluated 57 APS patients for antibodies to cardiolipin, β_2GPI, and for LAC activity. Twenty-eight of these patients had arterial disease and 29 had a history of venous thrombosis. A significant correlation was found between antibodies which bound to oxLDL-linked β_2GPI and classic anti-β_2GPI titers in these patients (Pengo et al., 2008). However, although a link between APS and autoantibodies targeting a lipid-regulating moiety was observed, the subgroup of patients with arterial outcomes were still older and had more risk factors for atherosclerosis than the subgroup who

had only suffered venous events (Pengo et al., 2008). Furthermore, in a different study of patients with primary APS, conventional measurement of hyperlipidemia was a better predictor for arterial events than anti-oxLDL antibodies (Bećarević et al., 2007). Whether these two variables are entirely independent of each other in vivo, over time, is not known.

Progression of atherosclerosis is now recognized to involve chronic inflammatory processes in artery walls, so endothelial injury by oxidized lipoproteins is likely to be an important part of this process, lending credence to the hypothesis that oxLDL antibodies might be contributing to plaque pathology in some way. This has been substantiated by several studies which do link autoantibodies to β_2GPI, to the development or progression of atherosclerosis or arterial thrombosis in SLE (Vaarala, 2000; Lopez et al., 2003, 2004; Zampieri et al., 2005; Matsuura et al., 2006) with or without a complex formation between β_2GPI and oxLDL.

Another report found that decreased annexin V binding to endothelium caused by autoantibodies (specifically aCL) was correlated to IMT results in patients with SLE (Cederholm et al., 2005). Elevated lipoprotein (a) has been implicated in premature atherosclerosis and high levels of lipoprotein (a) have been described in both SLE and primary APS. Antibodies to oxidized lipoprotein (a) have also been found in these populations (Romero et al., 2000; Carvalho, 2008). Other autoantibodies that are common in patients with APS which have been implicated in atherosclerosis risk include antiprothrombin antibodies (Vaarala, 2000), antibodies to lipoprotein lipase (Vuilleumier et al., 2004), and antibodies to apolipoprotein A1 (Delgado et al., 2003). It seems likely that aPL could have significant indirect effects on lipid metabolism as will be further discussed below.

In summary, antibodies that are currently considered integral to the APS might contribute to atherosclerosis, but detection of their impact in smaller clinical studies with limited variables is complicated by the likelihood that an expanded panel of closely related autoantibodies that are frequently found in the same and/or overlapping patient populations is involved. Furthermore, the effect of autoantibodies can be direct, by interfering with plaque formation or stability, or indirect, through inflammatory mechanisms or effects on lipid metabolism or other traditional risk factors. For this reason studies that fail to implicate a direct effect of autoantibodies, while detecting some of the traditional risk factors which they might be affecting, may be underpowered to sort out the real impact of these autoantibodies. The same can be said for studies that have detected an association; they are simply underpowered to tackle all of the potential confounding variables. An obvious clinical example of this kind of confusing relationship between aPL and other clinical risk factors is provided by a report of high levels of homocysteine which has been related to the presence of LAC in patients with cerebral ischemia (Chen et al., 2007).

Autoantibodies do not always travel in simple diagnostic herds. Small subsets of antibodies that bind to a given target may behave differently from other subsets despite the fact that the clinician cannot tell them apart in a diagnostic assay. For example, in one study which demonstrated cross-reactivity between aCL and oxLDL, it was pointed out that only a minor subset of the aCL may actually cross-react (Damoiseaux et al., 2005). Some patients who test positive for aCL might have that subset circulating in their bloodstream, others not.

In light of these formidable variables, it is impressive that so many clinical associations have been found between aPL and atherosclerosis endpoints. Meanwhile, there is a growing literature supporting the potential for aPL (or related vascular autoantibodies) to affect key mechanisms in atherosclerosis progression, underscoring the probability that they act via indirect mechanisms, so that their effects might be inexorably intertwined with traditional cardiovascular risk factors.

4. Intravascular autoantibodies: evidence for effects on traditional cardiovascular risk factors

As discussed above, disturbance of blood vessel homeostasis by autoantibodies could occur by alternative routes. Antibodies might directly interfere with blood vessel-regulating proteins or they could

trigger intravascular inflammation. Indeed, atherosclerosis is recognized to be the net result of chronic blood vessel inflammation (Blake and Ridker, 2001; Pepys and Hirschfield, 2001). In fact, the model suggesting that atherosclerosis arises due to chronic disturbance of vascular homeostasis implies that some immune mechanisms may be integral to the regulation of healthy vascular processes.

T cell activation and the egress of monocytes into artery walls is an important step in cholesterol metabolism. However, excess T cell cytokines can inhibit expression of cholesterol 27-hydroxylase (Sorice et al., 1996). Thus the same immune-mediated events that regulate vascular homeostasis might, when there is excess inflammation, cause impairment of lipid metabolism pathways. aPL can target and interfere with many blood vessel elements, including endothelial cells, proteoliposomes, platelets, monocytes, and circulating coagulation factors (Galli, 1996). In the process of antigen–antibody interactions and Fc receptor interactions, inflammatory events may be triggered, further imbalancing vascular regulation (Asherson et al., 1991; Davis and Brey, 1992; Holers et al., 2002).

Atherosclerosis involves complement activity (Halkes et al., 2001; Pepys and Hirschfield, 2001; Yasojima et al., 2001a, b). C-reactive protein (CRP), a complement activating protein, is an established marker for cardiovascular risk in patients with acute coronary syndromes and healthy people (Pepys and Hirschfield, 2001). High-sensitivity CRP assays were developed when it became apparent that CRP levels that were previously considered normal were high enough to increase risk of plaque progression, illuminating the potential subtlety of some cardiovascular risk factors. Other complement components, such as sC5 b-9, are elevated in patients with hypercholesterolemia and atherosclerosis, and inversely related to high-density lipoprotein (HDL) (Pasqui et al., 2000). CRP has binding specificity for low-density lipoproteins as well, suggesting that appropriate levels of CRP could have a healthy role in lipid metabolism (Pepys and Hirschfield, 2001). Since complement activity might have both positive and negative effects on blood vessels, it has been speculated that treatment should aim to modulate inflammation rather than to abrogate it (Chakraborti et al., 2000).

Autoantibody-mediated complement activation is a classic feature of autoimmune disease. Complement activation may also be involved in thrombosis, as observed in an animal model of the APS (Holers et al., 2002). Complement activation has also been associated with events of the APS in humans (Asherson et al., 1991; Davis and Brey, 1992) and with in vitro procoagulant activities of aPL (Stewart et al., 1997). In a study of brain ischemia, abnormal complement was associated with the subset of patients who had aPL (Davis and Brey, 1992).

Direct binding of pathologic antibodies to many structures in the vascular could arise by autoantibody spreading and/or molecular mimicry. Endothelial dysfunction is now an accepted risk factor for the progression of atherosclerosis. Endothelial cells may have epitopes that are similar to structures on β_2GPI and oxLDL (Wu et al., 1999). Oxidation of lipids in endothelial cell membranes could provide a permissive environment for autoimmune atherogenesis, and therefore it is not surprising that IgG aCL have been associated with endothelial dysfunction in a study of diabetes patients (Ciarla et al., 2001). Animal models have provided a more direct proof of concept to implicate aPL in mechanisms of endothelial disfuntion. Passive transfer of monoclonal aPL from a model with APS and coronary artery disease resulted in endothelial dysfunction in the recipient mice as measured by reduction in the acetylcholine relaxation after phenylephrine contraction (Belizna et al., 2008b). For these reasons, a multivariate analysis that models both aCL and endothelial dysfunction as independent variables in plaque formation might fail to pick up the impact of aCL on endothelial dysfunction, especially since the two might be partially independent via other mechanisms.

The evidence of autoantibody interference with structures critical to lipid metabolism is growing. The enzyme cholesterol 27-hydroxylase is found in arterial endothelial cells and in monocytes, which solubilizes cholesterol to promote its removal from arterial walls. Reiss et al. found that interferon gamma and immune complexes bound to complement decreased expression of cholesterol 27-hydroxylase in human aortic endothelial cells and monocyte lineages (Reiss et al., 2001).

Downregulation of cholesterol 27-hydroxylase by immune complexes was also dependent on complement fixation (Reiss et al., 2001), again demonstrating the co-dependence of many identified variables in an accelerated atherosclerosis mediated by autoimmunity. Elevated lipoprotein (a) has also been implicated in premature atherosclerosis, High levels of lipoprotein (a) have been described in both SLE and primary APS, and antibodies to oxidized lipoprotein (a) was also described in these populations (Romero et al., 2000). Reichlin et al. have identified autoantibodies to lipoprotein lipase in an autoimmune population with a correleation between these autoantibodies and fasting triglycerides, apolipoprotein B, and apolipoprotein E concentrations (Reichlin et al., 2002), suggesting that these might not be completely independent variables for atherosclerosis when autoimmunity is involved. The presence of these autoantibodies in a lupus population has also been confirmed by a different group (de Carvalho et al., 2004).

Lahita and colleagues reported low levels of the cholesterol transport protein apolipoprotein A1 (apoA1) in lupus patients, and subsequently specific antibodies to purified apoA1 were identified in lupus patients and in 22.9% of patients with primary APS (Lahita et al., 1993; Merrill et al., 1995). A link between these antibodies and markers of inflammation is suggested by finding anti-apoA1 autoantibodies in 1% of a healthy control group, 21% (11/53) in APS patients, 13% in SLE patients (Dinu et al., 1998). The titer of anti-apoA1 in a different APS population was correlated to both plasma apoA1 levels and amyloid A concentration (Vuilleumier et al., 2004). One report suggests that there may be cross-reactivity between autoantibodies to cardiolipin, HDL, and apoAI in autoimmune patients (Delgado et al., 2003). Anti-apoA1 were also associated with but not necessarily cross-reactive to anti-β_2GP1, and bound best to apoA1 when embedded in proteoliposomes reconstructed to resemble mature HDL molecules (Dinu et al., 1998). Thus the active composition of lipids and proteins in HDL may be critical to the effect of autoantibodies on risk for atherosclerosis, raising the possibility that these autoantibodies might increase risk in some of the patients some of the time, but not in all of the patients, and not all of the time.

Delgado Alves and colleagues have reported that autoantibodies targeting HDL and β_2GPI are associated, both in patients with SLE and those with primary APS, with reduced activity of paroxynase, an enzyme which prevents lipoprotein oxidation (Delgado et al., 2002). Based on broad associations between oxidative stress and APS, which will be further discussed below, the effects of aCL on the oxidation state of BALB/c severe combined immunodeficiency (SCID) mice was explored. Paroxynase activity and nitric oxide were reduced in animals injected with monoclonal aCL but not control antibodies produced in the same hybridoma system (Delgado et al., 2005). It seems possible that direct interference of these autoantibodies with paraoxonase activity, an HDL-related antioxidant enzyme, could contribute to the oxidative stress found in these conditions.

Oxidized LDL has been strongly implicated in atherosclerosis, and cross-reacts with aCL (Wu et al., 1999) One study using a murine model for the APS showed cross-reactivity between the aCL from these mice and oxLDL (Mizutani et al., 1995). β_2GPI is now known to bind to oxLDL, and is recognized by autoantibodies in that structure, which could lead to an alternative, Fc-mediated uptake of LDL by macrophages (Hasunuma et al., 1997; Koike, 2000). This raises the possibility that aPL can directly contribute to formation of atherosclerotic plaque. In fact it has been observed that β_2GPI inhibits uptake of oxLDL by macrophages, suggesting a role for it as an anti-atherogenic protein, potentially inhibited by antiphospholipid-related autoantibodies (Hasunuma et al., 1997). Validation of this concept has been provided by animal studies. In a murine model in which a lupus-susceptible strain was combined with a mouse deficient in LDL receptors, the lupus–atherosclerosis model offspring developed both lupus-like disease and accelerated atherosclerosis. The plaques of these mice demonstrated inflammatory pathology and increased antibody to oxLDL and cardiolipin (Stanic et al., 2006). Combination of lupus murine models with the apoE knockout mouse model for atherosclerosis resulted in increase of atherosclerosis (Ma et al., 2008), increases in anti-oxLDL and aCL. In a passive transfer model, LDL receptor knockout mice immunized with IgG from patients with APS

developed aCL and accelerated fatty streak formation (George et al., 1997). Adoptive transfer of T cells that react to β_2GPI-accelerated fatty streak formation in mice that were deficient for the receptor of LDL (George et al., 2000). In fact, β_2GPI has been identified in human atherosclerotic plaques (George et al., 1999), as would be likely to happen in states of excessive macrophage uptake of the complex of this protein with LDL. Once there, it could serve as a target for further autoimmune responses that could influence plaque progression (George et al., 1999).

When β_2GPI forms complexes with oxLDL specific autoantibodies can arise in some subsets of patients targeting a specialized ligand that develops within this complex. In a study of 150 patients with SLE and/or APS, the levels of antibody to this ligand were significantly higher in patients with the APS than those with SLE but without APS and/or than healthy controls (Lopez et al., 2003). More importantly, the levels of autoantibodies to this ligand of APS patients with arterial thrombosis were significantly higher than APS patients with only a history of venous thrombosis and/or pregnancy morbidity. Thus, oxidation of LDL might lead to complex formation with β_2GPI both in SLE and in patients with the APS, but specific autoantibodies against a specialized ligand that develops in this complex were more strongly associated with the subset of patients who had arterial thrombosis. In a different study, antibodies to oxLDL were associated with venous, but not arterial thrombosus (Hayem et al., 2001), however that study did not look at the same specificity described above, and was also underpowered to have detected a clinically significant association with arterial risk.

Under some circumstances, it can be hypothesized that autoantibody-induced permissiveness for uptake of oxLDL by macrophages could be beneficial to a host, providing a temporary extravascular reservoir for blood lipids when HDL is absent or impaired. In this model, only when macrophage uptake of LDL becomes prolonged or excessive would this promote plaque formation in arterial walls. This would provide another level of complexity to the clinical analysis of whether aPL promote atherosclerosis. Previously it has been considered possible that autoantibodies might indirectly promote traditional risk factors that are currently being modeled as independent variables in clinical studies. It has also been considered likely that only subsets of autoantibodies (for example only those that bind selected ligands on a structure) are pathogenic. The data reviewed above do not rule out the additional layer of complexity that under some circumstances autoantibodies may play a beneficial role in immune-mediated vascular homeostasis.

Such autoantibodies, which should be tightly regulated by the immune network in healthy people and released into the bloodstream only to provide balance during temporary vicissitudes in vascular homeostasis, are termed "natural" autoantibodies, and their potential impact on atherosclerosis progression in healthy people or people with the APS will be considered in the next section.

5. Natural autoantibodies: can they protect against atherosclerosis and are they related to antiphospholipid antibodies?

There is a limited repertoire of "natural" autoantibodies, which tend to bind to protein sequences that are redundant in nature and conserved in evolution (Czompoly et al., 2006), supporting a benign purpose for these autoantibodies (Baumgarth et al., 2005; Chang et al., 2005; Binder and Silverman, 2005; Czompoly et al., 2006). In fact, autoantibodies can frequently be detected in blood samples from healthy people by special methods, their usual detection masked by conventional testing methods (McIntyre, 2004; Blank et al., 2004). Anticardiolipin antibodies can be found in approximately 12% of a healthy elderly population and 2% of a younger population by usual techniques (Fields et al., 1989; Shi et al., 1990) but are markedly increased when chemical treatments are used to "unmask" autoantibodies that are bound up by regulatory networks of "anti-idiotype" autoantibodies. Anti-idiotypes are thought to be another group of natural autoantibodies which specifically target the active sites of other antibodies, presumably rendering them harmless until needed (Blank et al., 2004; McIntyre, 2004; McIntyre et al., 2005, 2006).

Natural autoantibodies and many aPL seem to share a "clean-up" role in the vasculature, by binding to membrane structures on dying cells (apoptotic cells) and facilitating their clearance (Czompoly et al., 2006). The cell membranes on apoptotic cells create oxidation-specific epitopes that attract both natural antibodies and aPL (Chang et al., 2005; Binder and Silverman, 2005; Czompoly et al., 2006; Stahl et al., 2006), supporting the recurrent theme of dysregulated oxidation in the APS and in inflamed vasculature, and promoting the hypothesis that atherosclerosis could be accelerated in inflamed vasculature when this clean-up process becomes inefficient or otherwise impaired.

Evidence for this "yin-yang" of antibodies is growing, since not only can autoantibodies be unmasked in healthy people by chemical treatments, but antibodies that are initially found in sera of patients with autoimmune diseases can be rendered invisible after similar treatments (McIntyre et al., 2005, 2006). In fact, various chemical changes can also lead to simultaneous masking and unmasking of different autoantibodies in one serum sample, suggesting that oxidation or other inflammatory changes in blood vessels might cause small modifications to how antibodies bind to their targets, potentially disturbing the homeostasis of controlled release of natural autoantibodies, rendering those usually benign proteins more pathogenic (Yuste and Prieto, 2003; Asherson and Cervera, 2003; McIntyre et al., 2005).

By an extension of this logic, whether antibodies contribute to atherosclerosis might depend on how and when they bind to intravascular structures, what other variables may be disturbing the intravascular milieu and how the specific structural–functional relationships of these structures is affected in the process. It may be not only that specific subsets of autoantibodies are benign and others pathogenic, but that this outcome can be reversed in permissive intravascular conditions.

Some evidence supports the idea that autoantibodies associated with the APS develop from natural autoantibodies for purposes that are beneficial to host defense, and only become deleterious when there is dysregulation of the vasculature. First, aPL, including those listed as vascular regulators in Table 2, are similar to natural autoantibodies by their tendency to bind strongest to structures in oxidized environments (Rauch et al., 2000). Second, small mutations of an aPL has produced a non-pathogenic autoantibody that maintained ability to bind self structures but was no longer pathogenic (Lieby et al., 2004). In another report, a patient with multiple antibodies binding different structures on β_2GPI and prothrombin was studied (Languren et al., 2006). Using a phage display approach, two antibodies were found that reacted with β_2GPI and one that recognized prothrombin and cross-reacted with β_2GPI. All three clones recognized anionic phospholipids (Languren et al., 2006). The antibody specific for β_2GPI contained highly conserved germline genes, suggesting it was a natural autoantibody. The one that cross-reacted with prothrombin seemed to contain antigen-dependent mutations in the antigen-binding areas of the antibody, suggesting a more classic, and potentially more pathogenic autoantibody. Taken together, these reports suggest a model for autoimmunity in which beneficial natural autoantibodies, genetically predetermined as part of host survival, might, under certain circumstances, begin to mutate in directions leading to pathologic autoimmunity. An alternate model suggests the opposite possibility, in which defects in natural inhibitors of aPL may contribute to pathogenicity by allowing excessive exposure of these antibodies in the bloodstream. In this construction, these antibodies might be helpful during temporary fluctuations in blood vessel homeostasis, but cause disease when allowed to circulate for too long (Lieby et al., 2004).

That fundamental vascular homeostasis involves interactions of natural antibodies and oxidation

Table 2
Antiphospholipin antibodies and risk for atherosclerosis: controversy in the literature

Outcomes[a]	Subject number	Type of study	References
Positive associations	116	Cross-sectional	Galli (2004)
	300	Cross-sectional	Pengo (2008)
	546	Prospective	Shortell (1992)
Negative associations	125	Cross-sectional	Lopez (2004)
	394	Case–control	Medina (2003)

[a] Partial list to demonstrate examples.

epitopes from phospholipids is not a new concept, suggesting broad dynamic connections between host defenses, autoimmunity, and atherosclerosis (Binder and Silverman, 2005; Languren et al., 2006). In light of this it would seem unlikely that all aPL have the same deleterious effects all the time. A proof of concept for atherosclerosis-protecting autoantibodies in an animal model has been reported (Nicolo et al., 2003). In this study, a monoclonal aPL was produced from an autoimmune mouse which develops fatal myocardial infarctions. The antibody from this mouse was found, in vitro, to cross-react with both native and oxidized LDL. Passive administration of this antibody to a different mouse which was prone to atherosclerosis by knockout of its receptor for LDL significantly reduced plaque formation. Various reports of autoantibody specificities for LDL include some reacting only against oxidized forms of these lipoproteins, others that react with both reduced and oxidized forms (Nicolo et al., 2007), underscoring the variability in possibilities for pathogenic or protective roles for these autoantibodies.

In another study, natural antibodies to oxLDL found in atherosclerosis-prone mice have been proposed to be anti-atherogenic (Shaw et al., 2000). Differing effects have been described, comparing an antibody to β_2GPI and a natural autoantibody targeting oxLDL. The first autoantibody increased uptake by macrophases of β_2GPI bound to oxLDL, the natural autoantibody, which was, interestingly, derived from an atherosclerosis-prone mouse, inhibiting this pro-atherosclerosis process (Kobayashi et al., 2007). Fine-specificity assays suggest that patients with atherosclerosis make specific, targeted autoantibodies which recognize domain 4 of the β_2GPI molecule (Kobayashi et al., 2007). These epitopes are distinct from the majority of other autoantibodies found in APS samples which have been studied using the same domain-selective approach (Iverson et al., 1998).

Therefore, depending on the waxing and waning background physiology of a patient over time and the fine specificities of a given autoantibody, antibodies belonging the broader spectrum of the APS-associated repertoire could be either protective or increase the risk for atherosclerosis. In states of disordered immunity, the weight of the literature suggests that the net effect of a polyclonal immune response, waxing and waning over years, is unlikely to be fully protective. Whether better descriptions of natural autoantibodies, improved molecular understanding of intravascular antigen–antibody interactions under stable vs. inflamed conditions, and clever leveraging of information learned from animal models could reverse this outcome remains unknown, but seems theoretically possible.

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CHAPTER 15

Treatment of Thrombosis in the Antiphospholipid Syndrome

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1. Introduction

Thromboses are among the most severe manifestations of the antiphospholipid syndrome (APS), particularly those affecting cerebral arteries (Cervera et al., 2002). Thus, correct management of thrombotic complications is paramount to improving the prognosis of patients with antiphospholipid antibodies (aPL). In fact, the ideal approach would be to prevent patients with aPL from developing full APS, that is, to keep them asymptomatic. Unfortunately, on many occasions the clinical event comes first, and it is at that time when we notice the presence of aPL. We all agree that whatever the clinical scenario—patients with thrombosis or asymptomatic aPL carriers—every effort must be made to prevent clotting events. However, the optimal treatment regimes are sometimes a matter of intense debate.

Mirroring what usually occurs in the field of systemic autoimmune diseases, the initial studies in APS consisted of observational cohorts (Rosove and Brewer, 1992; Khamashta et al., 1995). It was after the publication of these series that the recommendation of prolonged (indefinite) anticoagulant treatment for patients with APS and thrombosis ensued. Over the following years, studies with higher quality have been published, including two randomized controlled trials (RCTs) (Crowther et al., 2003; Finazzi et al., 2005), a subgroup analysis of a RCT (Levine et al., 2004), and two systematic reviews (Lim et al., 2006; Ruiz-Irastorza et al., 2007).

Unfortunately, observational and experimental studies have often reached different conclusions. While RCTs are studies of higher quality, in this specific field they are often limited by difficulty recruiting large enough groups of patients to represent the whole spectrum of the syndrome. On the other hand, observational series, although methodologically weaker, have the potential advantage of the larger size and the inclusion of non-selected, "real world" patients. Indeed, important management issues involving patients with systemic lupus erythematosus (SLE) and other autoimmune diseases are based on observational studies (Urowitz and Gladman, 2005). Thus, any available information is potentially useful and the final conclusions must take into account both methodological and population-based limitations.

An important issue to take into account when making therapeutic decisions is the immunological profile of patients with aPL. Those with lupus anticoagulant (LAC) should be considered to be at the highest risk of thrombosis (Galli et al., 2003), particularly if concomitant high levels of anticardiolipin (aCL) or anti-β_2 glycoprotein I (anti-β_2GPI) are demonstrated (Forastiero et al., 2005). In patients with SLE, LAC and persistently positive aCL seem to be predictive of thrombosis, while occasional aCL, even if repeatedly positive, do not increase the thrombotic risk (Martinez-Berriotxoa et al., 2007).

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2. Prevention of recurrent thrombosis

In 2006, Lim and colleagues published a systematic review in JAMA that focused on the therapeutic aspects of APS (Lim et al., 2006). For patients with thrombosis, the authors recommended that patients with aPL experiencing a venous or non-cerebral arterial thromboembolism should be treated with oral anticoagulation at a target international normalized ratio (INR) of 2.0–3.0. However, those patients having a stroke should receive either low-dose aspirin or warfarin at a lower aimed INR (1.4–2.8). This systematic review limited the selection of papers to RCTs. Therefore, their recommendations for treatment were unsurprisingly based on only three studies (Crowther et al., 2003; Finazzi et al., 2005; Levine et al., 2004).

The studies by Crowther et al. (2003) and Finazzi et al. (2005) were well-designed RCTs with intention-to-treat analysis, comparing conventional (i.e. target INR 2.0–3.0) with high intensity (i.e. target INR 3.0–4.0) anticoagulant treatment in patients with APS and previous thrombosis. All patients had definite APS according to the Sapporo criteria (Wilson et al., 1999). No differences in the rate of recurrent events between the high and the standard intensity groups was found in any of these studies, leading to the conclusion that a target INR of 2.0–3.0 is as effective as a higher target value. However, some important biases have significantly limited the assumption of such a conclusion.

First, the rate of recurrent events was much lower than that expected, given the design and sample size calculations. In addition, the number of patients recruited by Finazzi et al. (2005) was below the calculated size. Both facts increase the probability of a beta error, which is of special concern in studies resulting in ''no difference between groups.''

Second, both trials failed to keep a number of patients randomized to an INR of 3.0–4.0, within the aimed intensity of anticoagulation. In the study by Crowther et al. (2003), this group was below the therapeutic range 43% of the time. Finazzi et al. (2005) did not report on this issue, but the mean INR in the high intensity group was 3.2, suggesting a significant number of measurements below the threshold of 3.0. However, the standard intensity groups had a good correlation between the target and the achieved INRs in both studies (Crowther et al., 2003; Finazzi et al., 2005). It is noteworthy that in the paper by Crowther et al. (2003), six out of the eight recurrent thrombotic events that occurred during the study period took place when the INR was actually lower than 3.0, whatever the therapeutic arm. In this setting, the intention-to-treat analysis can certainly be misleading.

Third, in both studies, patients with venous thrombosis accounted for 78% and 68%, respectively those recruited (Crowther et al., 2003; Finazzi et al., 2005). Patients with recent strokes were explicitly excluded from the trial by Crowther et al. (2003). In addition, both studies also excluded patients with recurrent thrombosis occurring during anticoagulant treatment (Crowther et al., 2003; Finazzi et al., 2005).

The third study selected by Lim et al. (2006) was the Antiphospholipid Antibody Stroke Study (APASS). This study was not a true RCT, rather a subgroup analysis of the WARSS (Warfarin Aspirin Recurrent Stroke Study), which was designed to compare the efficacy of antiaggregant and oral anticoagulant drugs to prevent vascular recurrences in the general population with stroke (Mohr et al., 2001). The APASS aimed to define the role of aPL in predicting recurrent strokes as well as predicting patients' response to therapy. Blood samples stored from 1770 patients (80% of WARSS participants) were tested for aCL (including IgG, IgM, and IgA isotypes) and LAC. However, aPL were tested on only one occasion. LAC or aCL were considered to be positive at any titer, including IgA aCL. Due to this lack of specificity, 41% of individuals in an unselected population of patients with stroke averaging 63 years of age were classified as aPL-positive. However, only 6.7% of patients were positive for both aCL and LAC, and just 0.2% had aCL at high titers. These results suggest that most aPL detected were probably transitory and incidental; in other words, only a minority of patients included in the APASS had APS.

Not surprisingly, the APASS did not find any relationship between aPL positivity and the risk of

recurrent thrombosis in patients with stroke, although the clinical course of those with concomitant positivity for LAC and aCL was worse. Moreover, the outcome of those aPL-positive patients was the same whether treated with low-dose aspirin or with oral anticoagulation to a target INR of 1.4–2.8. Unfortunately, these results, obtained in a study with such important limitations, were the only basis for the therapeutic recommendations for patients with aPL and stroke given by Lim et al. (2006).

In summary, the quality of the papers included in the systematic review by Lim et al. (2006) was limited by both the characteristics of the patients recruited, and the final achievement of the therapeutic goals in the RCTs. While the subgroup of patients with definite APS and venous thromboembolism is well represented in these studies, data were insufficient to recommend therapy for APS patients presenting with stroke. In particular, the results of the APASS could not be applicable to patients with definite APS.

We performed a second systematic review choosing a different approach. Observational cohorts were also included in the belief that, despite the intrinsic limitations of this type of study, they could add a broader clinical spectrum of patients with aPL. Sixteen papers were thus analyzed: nine cohort studies, five subgroup analyses, and two RCTs (Ruiz-Irastorza et al., 2007).

As expected, patients with only one positive determination of aPL did not behave differently from the general population, with a low incidence of recurrent thrombosis on oral anticoagulation or aspirin in this subgroup. Among patients fulfilling laboratory criteria for definite APS, the risk of recurrent events was lower in the cohorts of patients who predominantly presented with first venous thromboses than in the patients who presented with arterial and/or recurrent events. Standard intensity oral anticoagulation (i.e. target INR 2.0–3.0) protected the former from further thrombosis reasonably well. However, in the latter a better outcome was seen with higher intensity anticoagulation. It is noteworthy that amongst the events during which the actual treatment at that point was reported, only 3.8% of patients had an INR >3.0 (14% of all thromboses seen in patients treated with oral anticoagulants). Repeated thromboses were more frequent and were associated with a higher mortality than hemorrhagic complications in patients taking warfarin. Consequently, this review concluded that:

1. The absolute risk of recurrent events on oral anticoagulation is low for patients with venous thrombosis and aPL who do not fulfill laboratory criteria for definite APS.
2. Patients presenting with stroke with low titer aPL on one occasion do not have an increased risk of recurrent events compared with other stroke patients.
3. Among patients with definite APS, those presenting with a first venous event could be considered to be at a relatively low risk of recurrences while taking oral anticoagulation.
4. Patients with definite APS and arterial events or recurrent events are at a high risk of recurrences, even when treated with oral anticoagulation to a target INR 2.0–3.0.
5. Recurrences are infrequent among patients who are effectively receiving oral anticoagulation to an INR 3.0–4.0. Most thrombotic events in patients on warfarin take place at intensities below 3.0.
6. Among patients with APS, the risk of recurrent thrombosis was higher than the risk of major bleeding. Also, the mortality associated with thrombosis was much higher than that secondary to hemorrhages.
7. No data are available to clarify the role of aspirin combined with warfarin in resistant cases, or assessing the impact of correcting cardiovascular risk factors.

Taking into account the different risks of recurrences and response to treatment in patients with thrombosis and aPL, depending on the immunological profile and the type of thrombosis, the following recommendations for treatment were made (Table 1):

1. Patients with APS at low risk (first venous event) should be treated with warfarin at an INR of 2.0–3.0.

Table 1
Recommendations for secondary prophylaxis in patients with antiphospholipid antibodies and thrombosis

Diagnosis	Prophylaxis
Patients with definite APS and first venous event	Indefinite anticoagulation to a target INR 2.0–3.0
Patients with definite APS and arterial event	Indefinite anticoagulation to a target INR 3.0–4.0
Patients with definite APS and recurrent events	Indefinite anticoagulation to a target INR 3.0–4.0
Patients with venous thromboembolism with single or low-titer aPL	6 months anticoagulation to a target INR 2.0–3.0
Patients with arterial thrombosis with single or low-titer aPL	Low-dose aspirin

Note: APS, antiphospholipid syndrome; aPL, antiphospholipid antibodies.

2. Patients with APS and arterial thrombosis and/or recurrent events should be treated with warfarin at an INR >3.0.
3. Patients with venous thromboembolism or stroke with a single positive determination for aPL should have further aPL testing. If they have only one positive aPL then they should be treated no differently to the general population (warfarin to a target INR 2.0–3.0 and low-dose aspirin, respectively).
4. There are no data to recommend additional antithrombotic treatment such as aspirin for patients having recurrent events while on oral anticoagulants achieving a 3.0–4.0 target INR. Likewise, the impact of correcting cardiovascular risk factors has not been established.

The final message is that patients with a high risk profile need to be treated more aggressively. It must be taken into consideration that in patients with no particular risk factors for bleeding, fear of hemorrhage should not preclude the adequate level of anticoagulation.

We acknowledge that these conclusions and recommendations are currently based on studies with less than ideal qualities according to accepted standards, and therefore have the potential risks of missing information and reporting bias. In the light of well-designed studies, which ensure the exclusive inclusion of individuals with definite APS and in whom all the clinical subsets (first and recurrent, arterial and venous events) are adequately represented, these guidelines could be retrospectively changed.

3. Primary thromboprophylaxis

Given the severity of APS, the ideal approach would be to avoid the progression from asymptomatic aPL carriers to patients with the syndrome. Retrospective studies suggest that patients with SLE and aPL have a 50% chance of suffering APS manifestations within 10 years (Shah et al., 1998). A Spanish series has recently quantified the risk of thrombosis in this group of patients: 3.93 events per 100 patient-years (Martinez et al., 2006). Women with obstetric APS also seem to be at a high risk of thrombotic events, being at an even higher risk than patients with SLE (Erkan et al., 2001). On the other hand, purely asymptomatic aPL carriers may have a much lower risk (Girón-González et al., 2004).

Low-dose aspirin has long been used as primary thromboprophylaxis in patients with aPL, following retrospective studies (Erkan et al., 2001) and a Markov decision analysis (Wahl et al., 2000). However, a recent placebo-controlled RCT (the APLASA study) has failed to show a beneficial effect of aspirin in preventing thrombosis in asymptomatic patients with aPL (Erkan et al., 2007).

Once again, this study had important limitations. The authors excluded women with obstetric APS, and included patients with IgA aCL. The number of participants with LAC was not reported, even when that specific question was addressed in a letter to the editor (Wahl et al., 2008). Moreover, the inclusion of patients was concluded early due to difficulties in recruitment, thus the final population size was smaller than the calculated one.

Table 2
Primary thromboprophylaxis in patients with antiphospholipid antibodies

Diagnosis	Prophylaxis
Patients with SLE and LAC and/or persistently positive aCL	Hydroxychloroquine and low-dose aspirin
Patients with obstetric APS	Low-dose aspirin
Asymptomatic carriers of aPL	No therapy or low-dose aspirin
In addition, in all patients with aPL	Strict control of vascular risk factors
	Adequate thromboprophylaxis in high-risk situations (surgery, post-partum, prolonged immobilization)

Note: SLE, systemic lupus erythematosus; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies.

Whether or not a result of the above limitations, the final frequency of events was very low in both the placebo and aspirin groups. Accordingly, aspirin was not shown to prevent thrombotic events. However, the results could be different in a population with a higher risk profile. Indeed, an interesting finding of this trial was that most events happened in patients with either a connective tissue disease or who had concomitant risk factors.

Despite the results of the APLASA study, our recommendation is to give aspirin to patients with SLE and persistently positive aPL (especially to those with LAC), and to women with obstetric APS. Hydroxychloroquine could offer an additional protection in patients with lupus (see below). We do not routinely recommend aspirin to asymptomatic individuals with aPL. However, those with concomitant LAC and high-level aCL and/or anti-β_2GPI could also be candidates for primary thromboprophylaxis. In addition, any carrier of aPL, whether or not they have had a previous thrombosis, should have a strict control of vascular risk factors and an adequate prophylaxis of venous thromboembolism in high-risk situations (Table 2).

4. Other therapies

Animal and in vitro models show an antithrombotic effect of hydroxychloroquine, reducing the size and the time of persistence of aCL-induced clots in a mouse model (Edwards et al., 1997) and in aPL-induced human platelet activation (Espinola et al., 2002), with both effects taking place in a dose-dependent fashion.

Earlier, Wallace (1987) published the observation that lupus patients taking antimalarials were less likely to develop thrombosis (45.5% of patients with thrombosis took hydroxychloroquine, vs. 77.8% of the complete cohort). The same was true for those with aCL (Wallace et al., 1993). Similar results were observed in the Hopkins Lupus Cohort (Petri, 1996a, 1996b). A cross-sectional study by Erkan et al. (2002) compared 77 patients with APS and thrombotic events with 56 asymptomatic aPL carriers, with or without SLE. The analysis of potential risk factors and protective factors against thrombosis which were present within the six months previous to the index event found that hydroxychloroquine use decreased the risk of thrombosis. The effect was similar to aspirin in both bivariate and logistic regression models.

More recently, we studied the specific role of antimalarials in preventing thrombosis in a prospective lupus cohort of 232 patients (Ruiz-Irastorza et al., 2006). Forty-two thrombotic events were seen after the diagnosis of SLE. Of these, only seven (17%) happened while the patient was taking antimalarials; an additional seven episodes (17%) took place after the patient had already stopped antimalarials, while the remaining 28 events (66%) occurred in 24 patients who had never received antimalarials. In a Cox multiple-failure time survival analysis, which analyzed treatment with antimalarials as a time-dependent variable, it was shown that antimalarials were an independent protective factor against thrombosis (hazard ratio (HR) 0.28, 95% confidence interval (CI) 0.08–0.90). In the same study,

aPL-positivity (HR 3.16, 95% CI 1.45–6.88) and previous thrombosis (HR 3.85, 95% CI 1.50–9.91) increased the risk of subsequent thrombotic events, as expected. Of note, no patients taking antimalarials died of cardiovascular causes.

Although we are still lacking specific studies in patients who have aPL without SLE, hydroxychloroquine is a firm candidate to complement anticoagulant therapy in patients with primary APS, given its excellent safety profile and the data supporting its antithrombotic effects. In our practise, we empirically add hydroxychloroquine to the treatment of patients with APS in whom a high-intensity INR cannot be achieved or in whom a high risk of bleeding precludes a target INR above 3.0.

Tissue factor (TF) is the most powerful in vivo inducer of thrombosis. There is experimental evidence that aPL upregulates TF expression in monocytes and endothelial cells (Lopez-Pedrera et al., 2006). Fluvastatin has been shown to inhibit such aPL-mediated increases in TF both in a mouse model (Ferrara et al., 2003) and in cultured human endothelial cells (Ferrara et al., 2004). Moreover, statins have also prevented the increased adhesiveness of endothelial cells induced by anti-β_2GPI antibodies (Meroni et al., 2001). Indeed, some authors suggest that statins will join the usual pharmacological armory for APS in the near future (Brey, 2004).

Finally, rituximab, an anti-CD20 chimeric monoclonal antibody that selectively induces a depletion of circulating B lymphocytes, has been shown to decrease aPL levels (Erre et al., 2008). Observational data suggest that this drug can be effective in aPL-induced immune cytopenias (Erdozain et al., 2004; Trappe et al., 2006) and recurrent thrombosis (Ahn et al., 2005). Thus, rituximab can offer a therapeutic alternative to severe cases of APS or patients with insufficient response to treatment.

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CHAPTER 16

Treatment of Infertility and Early Pregnancy Loss in the Antiphospholipid Syndrome

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1. Introduction

Clinical management of the gyneco-obstetric manifestations of the antiphospholipid syndrome (APS) is limited by diagnostic problems and a lack of carefully conducted randomized clinical trials. Thus, despite the enormous amount of work on the APS, there are still a number of questions regarding the management of reproductive failure potentially associated with antiphospholipid antibodies (aPL). This chapter outlines the current knowledge on the treatment of infertility and early pregnancy loss in patients with these antibodies.

2. Management of infertile patients having antiphospholipid antibodies

Pregnancy wastage associated with aPL may be due to multiple pathophysiologic factors entailing thrombotic phenomena, cytokine dysfunction, impaired embryonic implantation, aberrant placental hormone secretion in very early pregnancy, and complement activation, among others (Cervera and Balasch, 2008). Therefore, it has been postulated that there is a potential role for aPL not only in recurrent abortion but also in unexplained infertility and failure of nidation after in vitro fertilization (IVF) and embryo transfer.

On the other hand, the medications used for ovarian stimulation in IVF cycles cause a 10- to 20-fold increase in estradiol serum levels with respect to those found in a normal menstrual cycle. Thus, clinicians may be concerned about the potential adverse effects of these supraphysiological levels of estradiol on the clinical status of women with autoimmune disease. For APS, a primary concern would be that of thrombosis or embolism.

2.1. Treatment of infertility in patients having antiphospholipid antibodies

One of the greatest controversies in the field of autoimmunity and reproduction in recent years has been whether the presence of aPL has a role in the pathogenesis of female infertility, thus influencing reproductive outcome in infertile women undergoing IVF (Carp and Shoenfeld, 2007). In fact, several laboratories offer panels of serum autoantibody assays to screen women with unexplained infertility, thus implying that the results of the testing would alter clinical management. However, most studies (Balasch et al., 1996; Hatasaka et al., 1997; Coulam et al., 1999; American Society for Reproductive Medicine, 1999; Hornstein et al., 2000) have been unable to find differences in aPL-positivity rates between infertile women and

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fertile controls as well as between different diagnostic categories of infertile female patients, including unexplained infertility. In addition, a report investigating the possible association between aPL and spontaneous abortion after the first IVF and embryo transfer treatment has shown low and similar aPL-positivity rates in abortion (4.8%) and term pregnancy (4.8%) groups, thus indicating that aPL testing should not be considered in an infertile general population reaching an IVF program (Balasch et al., 1998). A meta-analysis of seven eligible studies on aPL and IVF outcome involving 2053 patients, of whom 703 (34%) had at least one abnormal aPL result, showed that there was no significant association between aPL and either clinical pregnancy or live birth (Hornstein et al., 2000).

Additional evidence for the lack of association between the presence of aPL and female infertility comes from therapeutic studies performed on aPL-positive infertile women undergoing IVF. The first such study (Birkenfeld et al., 1994) employed the use of 80 mg of aspirin daily and 10 mg of prednisone daily starting 2 weeks before initiation of IVF cycles. Subjects were 15 women who had failed to conceive during their latest embryo transfer cycle using either fresh or frozen embryos and who had presented at least one positive result for lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), or antinuclear antibodies at some unspecified time in the past. Seven (47%) women established ongoing pregnancies. The non-randomized study design, the lack of any controls, and the small number of patients preclude any comment about efficacy of treatment.

In another non-randomized study (Kutteh et al., 1997), the authors found that 9.9% of 191 infertile women undergoing IVF had aCL and an additional 8.9% were positive for other aPL. Nineteen women with antibodies were empirically treated with heparin and aspirin and 10 achieved pregnancies (52.6%), while 8 of 17 (47%) untreated women achieved pregnancies. These differences were not significantly different. In a third study (Schenk et al., 1996), the authors found that 43 of 90 (48%) IVF patients were positive for two or more aPL in a panel of immunoassays testing for IgG and IgM antibodies to six phospholipid antigens. These women were treated with heparin (5000 U subcutaneously, twice daily) and low-dose aspirin (81 mg, orally, daily). The per embryo implantation rate of 12.9% in the treated, seropositive women was not significantly different than in the untreated, seronegative women (7.7%). Moreover, neither the clinical nor ultimate ongoing pregnancy rates differed according to aPL status or treatment.

Finally, in the largest treatment study (Sher et al., 1994), the authors found a higher prevalence of aPL in women with confirmed pelvic pathology (pelvic inflammatory disease, iatrogenic abdominal/pelvic adhesions, or endometriosis) than in those with unexplained infertility. For treatment (heparin, 5000 U subcutaneously, twice daily and 81 mg of aspirin orally, daily), only selected women with pelvic pathology were included. Although how women were selected for treatment is unclear, 49% of those treated had clinical pregnancies compared with 16% of the 25 women not treated ($p < 0.05$). The relatively high rates of aPL in women with pelvic pathology differ from those of many other studies where no increase in aPL in women with tubal factor infertility was found. It is also crucial to recognize that a 49% clinical pregnancy rate in the aPL-positive, treated women is similar to the results of most experienced centers for comparable young patients (tubal factor, endometriosis) who are not treated with heparin and aspirin, whereas the reported 16% clinical pregnancy rate for the untreated patients in the same good prognosis categories is low compared to that in most programs. Also, when pregnancy rates of untreated aPL-negative and aPL-positive women were compared, the confidence limits of the ratio of the pregnancy rates (aPL-negative/aPL-positive) were 0.58–4.72, suggesting that aPL status had little effect on IVF outcome. Thus, it is possible that the apparently improved IVF pregnancy rates seen in treated infertile patients was independent of aPL status. An additional reason for these discrepancies among the studies could be the different cut-offs for aPL-positivity.

On the basis of the above evidence, it is well accepted at present that aPL are not related to female infertility, do not affect IVF success, and therapy for aPL is not justified.

2.2. Management of the risk of thrombosis in aPL-positive patients undergoing ovulation induction for IVF

The existing literature regarding the use of ovulation induction protocols in women with APS is scant, and definite conclusions about the risk of thrombosis cannot be drawn (Udoff and Branch, 2000). Nonetheless, it appears that the absolute risk of thrombosis is slight or modest at most. We have treated with ovulation induction over 20 infertile patients having aPL and none had a thrombosis in association with their treatment (Balasch et al., 1996, 1998). This may be due to the relatively short duration of elevated estrogens, the nature of the increased estrogens (estradiol and not a synthetic estrogen), and the hemostatic (physiological) milieu engendered by ovulation (natural or otherwise), which may differ from that in other circumstances.

One of the most difficult issues is how to manage heparin during ovarian stimulation for IVF. Since several treatment cycles may be required to achieve a pregnancy, the patient may be exposed to the risks of heparin over a course of 3–6 months. Also, the ovum retrieval poses a risk of ovarian bleeding in women on anticoagulant drugs. Based on existing information and the methods of ovulation induction and assisted reproductive technology employed, it is recommended that women with aPL and a history of thrombosis should be switched from oral anticoagulants to heparin for ovulation induction in IVF cycles. The heparin should be discontinued 12–24 hours prior to ovum retrieval and restarted 6–8 hours after ovum retrieval when the patient is clinically stable (Udoff and Branch, 2000).

3. Management of early pregnancy loss in patients having antiphospholipid antibodies

There are still a number of controversies regarding management of early pregnancy losses potentially associated with aPL. In this chapter we will discuss two of these questions: which patients with aPL should be treated and what treatment to use.

3.1. Patient inclusion criteria for treatment

Despite the proposals defining appropriate criteria for the classification of the APS (Miyakis et al., 2006), difficulties and differences in patient selection between studies may exist for several reasons: definition of the recurrent pregnancy losses is variable in the literature, inclusion of patients in the study group according to the aPL titer is author-dependent, and the assays for aPL are not well standardized. Recommendations for standardization of aPL assays have been published (Coulam et al., 1999; Greaves et al., 2000) but this subject is beyond the scope of the present chapter.

3.1.1. Pregnancy loss definition

According to the classification criteria of the APS (Miyakis et al., 2006), the cut-off between abortion (embryonic or early pregnancy loss) and fetal death (late pregnancy loss) is established at 10 weeks' pregnancy. Fetal death thus refers to the loss of a conceptus known to be alive at 10 weeks' gestation. So that the pregnancy was recognized alive after 10 weeks implies (1) the expulsion of a fetus measuring more than 30 mm; (2) in utero ultrasonic measurements compatible with a fetus; or (3) a miscarriage after the cardiac activity was objectified after the 10 th week (Branch and Silver, 1996; Vinatier et al., 2001; Derksen et al., 2001).

However, the interpretation of the patient's clinical history is usually difficult. Patients often report a miscarriage towards 10–12 weeks (when the signs of imminent abortion appear) whereas in fact the pregnancy has been demised for several weeks. This means that many expulsions considered in the fetal period are, in fact, of embryonic nature. This is important considering that: (a) different causes of abortion, including autoimmune factors, are known to result in pregnancy loss in any trimester of pregnancy in the same couple (Harger et al., 1983) and (b) second and third trimester fetal deaths have been considered to be more specific for APS (Lockshin, 1999).

In contrast with the above proposals, we and others (Balasch et al., 1990, 1996; Lockshin, 1999;

Greaves, 2000; Vinatier et al., 2001) agree that screening for aPL could be usefully extended to include women who do not fulfill the above strict criteria for APS but who have repeated miscarriage defined as two spontaneous abortions or three or more non-consecutive miscarriages. From a practical point of view this is a useful definition because it is clinically important to study and treat patients with a history of two or more miscarriages (Balasch et al., 2002).

3.1.2. Antiphospholipid antibody titer and isotype

The clinical significance of low-titer aCL in patients presenting with clinical features of the APS is considered to be doubtful. Thus, in cases where the aCL titer is less than 40 GPL units and tests for LAC are negative, it is considered that a diagnosis of APS may not be conclusive. Under these circumstances, it is recommended to consider other causes of thrombosis or miscarriage. There is also debate over the importance of the aCL isotype. IgG antibodies may be more clinically significant, although IgM aCL appears to be associated with thrombotic events and miscarriage in some series (Greaves et al., 2000). Tests for IgA antibodies may not be clinically informative (Selva-O'Callaghan et al., 1998) and their use is not currently recommended (Greaves et al., 2000).

The above notwithstanding, the clinical consequences of IgM aCL or low levels of IgG aCL remain controversial. This is a common dilemma for physicians, because patients with positive tests for aPL having either isolated IgM or low-positive IgG aCL are not uncommon (Pattison et al., 2000). Several facts indicate that the correlation between aPL and disease is rather imperfect (Balasch et al., 2002) and thus, treatment of subjects presenting with definite clinical criteria for APS but having low levels of aCL remains controversial in daily clinical practice. Considerations on prophylactic treatment of asymptomatic patients with aPL may help the physician in his or her decision-making process.

The aPL have been observed in 2–6% of apparently healthy blood donors and up to 30–40% of patients with lupus, yet the prevalence of thrombosis among these patients is very much smaller. Clearly, there are factors other than aPL that must be necessary for the full syndrome to develop. On the other hand, it is impossible to predict which patients with aPL will thrombose (Shah et al., 1998). The management of patients with persistently positive LAC or aCL without previous thrombosis have included taking low-dose aspirin indefinitely (Alarcón-Segovia et al., 2003). Similarly, whereas the prevalence of persistently positive tests for aPL in patients with recurrent abortion is around 10–15%, aPL are positive in 2–3% of unselected women of childbearing age and in more than one-third of systemic lupus erythematosus patients (Khamashta, 1998; Lockshin, 1999; Greaves, 2000). Even in normal pregnant women, the presence of antibody predicts a higher than normal loss rate for the current pregnancy. Nonetheless, since the frequency of abnormal tests is moderately high, and since incidence of fetal loss due to non-aPL causes is also high, positive predictive value is low (Lockshin, 1999). Thus, aPL-positive tests are not considered sensitive predictors of poor pregnancy outcome in women with no history of pregnancy complications (Greaves, 2000).

However, again there is no current test to predict which women with aPL will have pregnancy loss when becoming pregnant (Silver et al., 1996). Therefore, we agree with those authors stressing that low-dose aspirin is a safe option to offer primigravidas with positive aPL tests (Khamashta, 1998; Lockshin, 1999; Hachulla et al., 2000). The same principle would apply to women with a well-documented history of previous pregnancy losses and low titers of aPL or intermittent aPL (Lockshin, 1999). However, given that women with IgM only or low levels of IgG aCL comprise a population at less risk for aPL-related disorders, we also agree with those authors emphasizing that such patients should not be treated with potentially dangerous medications (prednisone, heparin) alleged to be efficacious for APS (Silver et al., 1996) since the balance between risk and benefit does not justifies therapy. Many clinicians recommend low-dose aspirin in women with low-titer aPL and the addition of heparin

if levels increase once pregnancy is confirmed (Kutteh et al., 1999).

3.2. Choice of treatment in antiphospholipid-associated early pregnancy losses

There have been some claims supporting the view that the presence of aCL in women with recurrent miscarriage may result in a modest increase in the risk of fetal losses and thus the chance of a successful pregnancy even without anticoagulant treatment is still favorable (Christiansen, 1997). However, there is now general agreement that whether or not aPL have a pathogenetic role, they serve as clinically important markers for risk in pregnancy. In any event, the uncertain etiology and pathogenesis of APS have meant that treatment has remained empirical and speculative. Our understanding of the possible mechanisms of action of aPL has led to two treatment modalities. The first one is focused on reducing the production of antibodies mainly with steroids and intravenous immunoglobulins. The second alternative includes the use of antiaggregant/anticoagulant agents, mainly aspirin and heparin, to prevent thrombosis in the uteroplacental circulation. Low-dose aspirin may also improve placental blood flow by decreasing the thromboxane-to-prostacyclin ratio. These therapeutic agents have been used alone or in combination.

Interventions such as these drug therapies and monitored pregnancy have increased fetal survival, but no gold standard has been determined. Available data are limited by the small number of patients in individual studies, which have also had varying entry criteria and treatment protocols, and by the lack of standardization in laboratory assays used to detect aPL.

Intravenous immunoglobulin treatment is still considered an experimental approach and its place for treatment of pregnant patients with aPL is still uncertain. Realizing the high and significant rate of preterm delivery and maternal complications existing with corticosteroid treatment, current therapeutic regimens emphasize various forms of anticlotting treatment in the form of aspirin and/or heparin.

3.2.1. Aspirin

Aspirin was nearly always added when corticosteroids were the mainstay of treatment. In addition to its effects on platelet aggregation and thromboxane–prostacyclin balance, low-dose aspirin has been found to significantly reduce the fetal resorption rate in the experimental APS (Krause et al., 1993). Aspirin inhibits irreversibly the synthesis of thromboxane A_2, a potent platelet aggregate. The assumption that cyclooxygenase inhibition is the mechanism responsible for the antithrombotic effect of aspirin can be further strengthened by the ability of BMS 180,291 (a highly selective and potent thromboxane A_2/prostaglandin endoperoxidase receptor antagonist) to reduce fetal resorption rate, to increase mean placental and embryo weights, to increase platelet counts, and to decrease activated partial thromboplastin time in mice with experimental APS (Shoenfeld et al., 2001). Data in the literature supporting the beneficial vasodilating effects of aspirin in conditions as diverse as intrauterine growth retardation with umbilical placental insufficiency to preeclampsia and thrombosis secondary to platelet aggregation, all support the idea that treatment with aspirin will help to prevent the vascular and thrombotic complications associated with aPL (Coomarasamy et al., 2001).

Good results with low-dose aspirin alone, with success rates over 70%, have been achieved by us (Balasch et al., 1993; Carmona et al., 2001) and others (Silver et al., 1993; Lima et al., 1996; Le Thi Huong et al., 2001) in APS patients with two or more pregnancy losses. Aspirin daily doses used in these studies ranged between 75 mg and 100 mg. The optimal antiaggregant dose for aspirin is still uncertain. Although doses as high as 325 mg three times a day have been used in the past, there is no evidence that doses higher than 75 mg/day are more effective in preventing thrombotic events, whilst toxicity is probably dose-related (Ruiz-Irastorza et al., 2001).

Potential complications of aspirin during pregnancy include birth defects and bleeding in the

neonate and in the mother. However, according to recent meta-analyses and large trials these potential effects on the mother and her infant appear at doses averaging 1500 mg/day, but not at doses $\leq$150 mg/day (Ginsberg and Hirsh, 1998). Thus, based on current evidence, low-dose aspirin ($\leq$150 mg/day) during pregnancy is safe for the mother and fetus. However, aspirin treatment has to be discussed in patients with abnormal platelet function, low platelet counts, or with hemorrhagic diseases.

3.2.2. Heparin

The use of heparin was a logical approach to treatment for a disorder resulting from thrombosis. In addition to its anticoagulant effects, heparin inhibits platelet function. Heparin has been reported to inhibit the binding of aPL to their target and to absorb aPL in vitro. Heparins are highly negatively charged molecules, and these in vitro effects are not surprising. It is not clear whether these effects are important in vivo in patients with aPL. No reports have shown that serum levels of aCL, for example, are decreased after acute treatment with heparin. In the earliest published case series (Rosove et al., 1990) it was observed that under heparin (mean dose 24 700 U/day), 14/15 pregnancies ended in live births in 14 women with aPL and history of 28/29 miscarriages. Over the past decade, several case series recounted a live-birth rate of $\sim$70–75% in women treated with unfractionated heparin, alone or in combination with low-dose aspirin (60–100 mg/day). Although some authors used sufficient doses to achieve full anticoagulation, equivalent results were achieved with prophylactic doses (Kutteh et al., 1999; Ruiz-Irastorza et al., 2001; Vinatier et al., 2001).

There is now accumulating experience with the use of low-molecular-weight heparins both in pregnant and in non-pregnant patients for the prevention of complications associated with aPL and there is also evidence that low-molecular-weight heparins do not cross the placenta and they are safe and effective in pregnancy (Sanson et al., 1999). A systematic review of the available evidence (Sanson et al., 1999) analyzed 486 pregnancies (163 with aPL and/or other autoantibodies) treated with low-molecular-weight heparins (nadroparin, enoxaparin, dalteparin, reviparin, and tinzaparin) as the only anticoagulant. This review demonstrated that this group of drugs are very effective, with only three cases of thromboembolic complications reported and no episodes of major bleeding. This is noteworthy considering that pregnancy is a high-risk period for thrombosis and all women with a previous history of thrombotic events must receive thromboprophylaxis with full anticoagulation during this period. Oral anticoagulants cross the placenta, are teratogenic (limited to chondrodysplasia punctata) and must, therefore, be avoided during the first trimester. The period of risk is between the sixth and twelfth week of gestation, so conception on coumadin derivatives is not dangerous provided that these drugs are replaced with heparin within two weeks of the first missed period. Therefore, in patients at high risk of recurrent thrombosis during pregnancy (those with previous arterial disease, mainly stroke), oral anticoagulants can be reintroduced in the second trimester if necessary (Ruiz-Irastorza et al., 2001). Coumadin is also used for postpartum thromboprophylaxis.

Low-molecular-weight heparins have potential advantages over unfractioned heparin during pregnancy because they cause less heparin-induced thrombocytopenia, have the potential for once-daily administration because of better bioavailability and longer half-life, and may result in a lower risk for heparin-induced osteoporosis. Prolonged heparin therapy in pregnancy has been associated with osteoporosis and vertebral collapse, especially when used in combination with prednisone (mainly at high dose) and thus some authors suggest that this combination should not be used (Kutteh et al., 1999). Administration of extra oral calcium (1000 mg/day) may help to minimize heparin-induced osteopenia.

3.2.3. Which drug to use?

The mainstay of treatment now rests with antiplatelet and antithrombotic treatments but the question of choice between heparin alone or with aspirin versus aspirin alone remains debated. No

considerable difference in terms of fetal outcome between pregnancies treated with only aspirin and those with heparin (alone or in combination with aspirin), whether associated with prednisone or not, has been observed (Balasch et al., 2002). However, this may be explained on the basis of selection criteria for heparin use which represents higher risk groups of patients because it is administered in cases of vascular history and/or when aspirin alone has failed in a previous pregnancy.

The current most recommended treatment for women with recurrent pregnancy wastage and aPL is heparin and low-dose aspirin, starting therapy when pregnancy is confirmed (Kutteh et al., 1999; Greaves et al., 2000; Derksen et al., 2001; Tincani et al., 2003). This recommendation is essentially based on two clinical trials which have found better obstetric outcomes using aspirin plus heparin than aspirin alone (Kutteh, 1996; Rai et al., 1997). The study by Rai et al. (1997) was a randomized trial, but Kutteh (1996) asigned treatment in a consecutive way, which limits the validity of his results. Results from both studies, however, were quite similar. Kutteh (1996) alternatively assigned aspirin (81 mg/day) or aspirin plus 10 000 U/day heparin in 50 women with aCL. The live-birth rate in the heparin-treated group was 80% vs. 44% in women treated with aspirin alone. Rai et al. (1997) compared aspirin with heparin 10 000 U/day plus aspirin in 90 women. The live-birth rate was 71% with heparin treatment versus 42% with aspirin alone. In both studies no differences were found between treatment groups with respect to obstetric complications. No case of thrombocytopenia or thrombosis occurred, but women receiving heparin plus aspirin had a median decrease in lumbar spine bone density of 5.4%. The potential limitations of these two studies have been previously stressed (Balasch et al., 2002).

It seems clear that aspirin has a place in the treatment strategy of early pregnancy losses associated with the APS and doubt as to whether heparin is always needed comes from the experience of several groups of investigators showing marked improvement in pregnancy rates during treatment with aspirin alone as compared with previous reproductive performance in the same women (Tincani et al., 2003). An important part of such improvement of prognosis in these patients is thought to be due to better obstetric surveillance. Indeed, a more recently published double-blind, randomized, placebo-controlled trial (Pattison et al., 2000), including 40 women with aPL and recurrent miscarriage, has not shown any benefit of adding aspirin to an intensive obstetric care and placebo treatment. The prognosis in both the aspirin and control groups was remarkably good, with success rates over 80%. However, it is noteworthy to note that treatment was started when pregnancy was diagnosed or on discovery of aPL during pregnancy but not before conception. Diagnosis of APS during gestation was an additional confounder in that study because maternal aPL may be downregulated during pregnancy and tests are best performed preconceptionally. On the other hand, most patients recruited for the study had only low-titer aCL and most important, emotional support and continuity of personnel were provided, including a liberal admission policy. Similar success rates with supportive care have been previously reported in women with unexplained recurrent miscarriage (Stray-Pedersen and Stray-Pedersen, 1984).

That study (Pattison et al., 2000) thus emphasizes a very important aspect in the management of these patients, and the only one where general agreement is found: that they should undergo close fetal and maternal surveillance by a well-coordinated multidisciplinary team including obstetricians, internists/rheumatologists, and hematologists.

In conclusion, because of the wide clinical diversity of the population with aPL, it appears illogical to treat all women the same. While in patients with a history of thrombotic events or an early pregnancy loss despite aspirin therapy the addition of heparin seems clearly indicated, we believe that, based on the evidence to date, heparin cannot be recommended for all women with APS and a history of previous recurrent early pregnancy losses. In the same way, aspirin alone may be insufficient for a number of patients even in the absence of thrombotic events. Therefore, despite the fact that the obstetric prognosis of APS has

been markedly improved by antithrombotic therapy, further studies are needed to determine the individual risks and the specific significance of the various aPL statuses and patient clinical profiles in order to better define the respective indications for aspirin and heparin alone or in combination.

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CHAPTER 17

Treatment of Late Pregnancy Complications in the Antiphospholipid Syndrome

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1. Introduction

The antiphospholipid syndrome (APS) is characterized by arterial and venous thrombosis and pregnancy complications that include recurrent early pregnancy loss and late adverse pregnancy outcomes such as fetal death, severe preeclampsia, placental insufficiency, and fetal growth restriction (Wilson et al., 1999; Levine et al., 2002) The pathogenic mechanisms that lead to injury in vivo are not clearly understood and evidence-based management of on-going pregnancies in women with APS and late pregnancy complications is difficult to provide, due to the lack of randomized clinical trials for this particular group of women. In this chapter, we outline some of the proposed mechanisms by which antiphospholipid antibodies (aPL) can cause and may perpetuate late complications of pregnancy. The identification of new mechanisms holds promise for new, more effective, and safer treatments. In addition, we focus on available therapeutic options for pregnant women with APS and late adverse pregnancy outcome.

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2. Obstetric complications associated with the antiphospholipid syndrome

In addition to recurrent spontaneous abortions before the 10th week of gestation and maternal thrombosis, the revised classification criteria for APS have reaffirmed preterm birth in association with severe preeclampsia/eclampsia, recognized features of placental insufficiency such as growth restriction or oligohydramnios, and late pregnancy loss as recognized clinical criteria for the diagnosis of this syndrome (Miyakis et al., 2006).

It is well established that a significant proportion of pregnancy losses secondary to APS are second or third trimester fetal deaths, with estimates of up to 50% in a cohort of 76 women with 333 pregnancies (Oshiro et al., 1996). Approximately one-third of women with APS will develop preeclampsia during pregnancy (Clark et al., 2007) and one study has reported a risk as high as 50% (Branch et al., 1992). This association is strongest in women with severe, early onset preeclampsia prior to 34 weeks of gestation. aPL may be particularly associated with a variant of severe preeclampsia, namely the HELLP syndrome, which is characterized by hemolysis, elevated liver enzymes, and thrombocytopenia (Ornstein and Rand, 1994; Dekker et al., 1995; Le Thi Thuong et al., 2005). Although the real incidence of HELLP syndrome in women with

APS is difficult to estimate, Le Thi Thuong et al. found that 53.3% (8/15) of women diagnosed with HELLP syndrome had APS.

Recently, a systematic review has positively identified aPL as one of the most significant predictors of preeclampsia (Duckitt and Harrington, 2005). Intrauterine growth restriction (IUGR) complicates 15–30% of pregnancies in women with APS (Branch et al., 1992; Caruso et al., 1993; Kutteh, 1996), although some studies have failed to find a correlation between aPL and IUGR (Lynch et al., 1994). Preterm delivery before 34 weeks of gestation may be indicated in around one-third of women with APS secondary to uteroplacental insufficiency, preeclampsia, or IUGR (Branch et al., 1992). In addition, these pregnancies are subject to other complications, such as systemic and pulmonary hypertension (Ruiz-Irastorza and Khamashta, 2007). In a case series of APS pregnancies that included women with systemic lupus erythematosus and a history of thromboembolic events, gestational hypertension or preeclampsia complicated up to 50% of cases. Additionally, placental insufficiency that necessitated delivery was a frequent complication (Lockshin et al., 1989; Caruso et al., 1993; Lima et al., 1996; Pauzner et al., 2001).

3. Mechanisms for adverse pregnancy outcomes

The mechanisms by which aPL could cause late pregnancy complications, such as preeclampsia, are not clearly understood. Given the association between aPL and arterial and venous thrombotic events, it is generally speculated that late pregnancy loss associated with APS is secondary to insufficiency in the blood supply resulting from massive thrombosis of the uteroplacental vasculature and subsequent placental infarction (Sebire et al., 2002; Rey et al., 2003). The fact that aPL are prothrombotic is widely supported in the literature. These antibodies can bind and activate endothelial cells and platelets, inhibit fibrinolysis, and even interfere with the protein C pathway (de Groot and Derksen, 2005). Furthermore, aPL have been demonstrated to cause reduction in trophoblast-associated annexin V levels, which may promote thrombosis at sites where fetal cells are exposed to maternal blood (Rand et al., 1997). Ogasawara and colleagues reported that β_2 glycoprotein I (β_2GPI)-dependent anticardiolipin antibodies (aCL) prevent the inhibitory effects of β_2GPI on factor X and thus induce uteroplacental blood insufficiency (Ogasawara et al., 1995). However, thrombosis does not to seem to be the only explanation for obstetric APS (Derksen and de Groot, 2008). In fact, thromboses in placentae and deciduas of women with APS may either be absent or present in insufficient quantities to account for pregnancy loss. Quenby et al. have recently demonstrated that aPL can inhibit extravillous trophoblast differentiation in vitro and have suggested that this, coupled with a failure of subsequent uteroplacental development, may play a part in the underlying pathology of aPL-associated pregnancy loss (Quenby et al., 2005). Recent experimental observations in mouse models, in which pregnant mice received human IgG containing either aPL or monoclonal aPL identified that activation of the complement cascade in the maternal–fetal interface is a crucial and key mediator of aPL-induced thrombosis and poor pregnancy outcomes (Girardi et al., 2003, 2006; Pierangeli et al., 2005; Xu et al., 2000). The local increase in complement activation fragments, particularly complement component C5 and its cleavage product C5a, causes dysregulation of angiogenic factors required for normal placental development. This has a highly deleterious effect on the developing fetus (Girardi et al., 2003, 2006; Holers et al., 2002).

Despite the lack of research in humans, this line of evidence may help us to understand the complex and wide range of APS manifestations, allowing the development of more targeted interventions to prevent late pregnancy loss and complications. A prospective, multicenter observational study is currently being undertaken to translate those findings to humans. The PROMISSE Study (Predictors of pRegnancy Outcome: bioMarkers In antiphospholipid antibody Syndrome and Systemic lupus Erythematosus) aims to evaluate the role of complement in aPL-induced pregnancy loss and fetal injury in women (Salmon and Girardi, 2008).

4. Therapeutic options for women with the antiphospholipid syndrome

The goals of treatment for APS during pregnancy are to reduce or eliminate the risk of thromboembolic events and to improve maternal and fetal outcome by reducing the risk of recurrent pregnancy loss, preeclampsia, placental insufficiency, and preterm birth. Since most therapeutic options entail risks for both the mother and the fetus, it is important that every option, with its pros and cons, is discussed in detail with the woman considering pregnancy in order to reach a joint decision. Treatment should be instituted only if the risk of aPL-associated complications exceeds that of the proposed treatment. The most widely used medications for treatment of pregnancy complications in women with APS are aspirin, heparin, warfarin, steroids, and immunoglobulins.

Most clinical trials evaluating the treatment of APS in pregnancy suffer from weaknesses. These include the lack of a uniform definition of pregnancy losses, lack of stratification by timing of losses (early vs. late), lack of evidence regarding whether treatments should be started pre- or post-conception, and when such therapy should be stopped prior to delivery. This is particularly true in the subgroup of women who have obstetric APS diagnosed based on prior fetal losses, or neonatal deaths after delivery at less than 34 weeks' gestation from severe preeclampsia or placental insufficiency. Most published treatment trials have included women with APS and recurrent first trimester pregnancy loss. The proportion of these women having had one or more prior fetal loss ranges from 11% (Kutteh, 1996) to 80% (Branch et al., 2000). Hence, treatment recommendations in this particular group are based on anecdote since these women have never been targeted in a randomized treatment trial to determine whether heparin or other therapies are efficacious and at what dose.

Whereas the treatment of women with APS and prior thrombosis is little debated, management in the absence of thrombotic events is still controversial. Most experts believe that women with previously confirmed thrombosis should be maintained on therapeutic-dose low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) in combination with low-dose aspirin throughout pregnancy and in the postpartum period (Bates et al., 2004; Ginsberg et al., 2001; Ruiz-Irastorza and Khamashta, 2007). Others recommend prophylactic-dose LMWH throughout pregnancy and the postpartum period, which is associated with an increased risk of recurrent thrombosis (Dentali and Crowther, 2006). If the patient was receiving warfarin derivatives prior to pregnancy, management consists of shifting to therapeutic LMWH as soon as pregnancy is diagnosed. If the patient is also receiving aspirin, it may be continued.

4.1. Aspirin

Aspirin reduces the risk of platelet-mediated vascular thrombosis by inhibiting thromboxane production. In women with APS, the question as to whether low-dose aspirin improves pregnancy outcomes has not been definitely answered (James et al., 2008) since most studies have included aspirin in both arms. Three trials have randomized aspirin in combination with heparin (Kutteh, 1996; Rai et al., 1997), or LMWH (Farquharson et al., 2002), vs. aspirin alone in the treatment of APS. Whereas the first two studies showed that combination therapy resulted in a higher rate of live births (71–80% vs. 42–50%) (Kutteh, 1996; Rai et al., 1997), the third study concluded that the addition of LMWH did not significantly improve pregnancy outcome (72% vs. 78%) (Farquharson et al., 2002). No trials have directly compared aspirin to heparin or LMWH. As a single agent for the treatment of aPL-associated pregnancy complications, there are at least three randomized trials that have compared aspirin vs. placebo or usual care in women with no previous thrombosis (Cowchock and Reece, 1997; Pattison et al., 2000; Tulppala et al., 1997). None found a benefit of aspirin although the third study included women with recurrent spontaneous abortions with or without detectable aCL (Tulppala et al., 1997). Combining the results of the three trials ($n = 71$) also showed no significant reduction in pregnancy

loss (relative risk (RR) 1.05, 95% confidence interval (CI) 0.66 to 1.68) (Empson et al., 2005). A common limitation of all three studies is that the small sample size made them underpowered to detect an improvement in the studied outcomes. For women with APS, there is consensus to start low-dose aspirin (75–81 mg daily) pre-conception and to continue it throughout pregnancy. Although it crosses the placenta, aspirin has not been associated with congenital malformations (Kozer et al., 2002; Norgard et al., 2005). However, several case series and epidemiologic studies have reported a possible association with gastroschisis due to an increased risk of vascular disruptions (Martinez-Frias et al., 1997; Torfs et al., 1996; Werler et al., 2002). Kozer et al. (2002), in a meta-analysis of 5 case–control studies, found a possible increased risk of gastroschisis in infants who were exposed to aspirin in the first trimester (odds ratio (OR) 2.37, 95% CI 1.44 to 3.88). Werler et al. (2002) also reported an elevated risk of gastroschisis with aspirin exposure (OR 2.7, 95% CI 1.2 to 5.9).

4.2. Heparin

As early as 1992, following the publication of the randomized multicenter trial by Cowchock et al. (1992), combination therapy with heparin and low-dose aspirin became the ''gold standard'' for treatment of APS in pregnancy. Fifty women were randomized to receive prednisone and aspirin vs. UFH and aspirin. Similar pregnancy success rates (75%) were noted in both arms, with a better safety profile in women receiving UFH and aspirin. At least three randomized trials were subsequently published in the English literature to establish whether heparin has an added benefit over aspirin alone in patients with recurrent pregnancy losses and APS (Farquharson et al., 2002; Kutteh, 1996; Rai et al., 1997) (also reviewed under ''Aspirin''). The superiority of aspirin/heparin was documented in two trials (Kutteh, 1996; Rai et al., 1997). A meta-analysis in 2002 suggested that in women with recurrent pregnancy losses, prophylactic heparin and low-dose aspirin may cause a 50% reduction in pregnancy loss (Empson et al., 2002). However, few data from controlled trials are available on the efficacy of various treatment regimens for women with APS and other obstetric complications such as fetal death, preeclampsia, and uteroplacental insufficiency. In general, most experts recommend prophylactic heparin and aspirin during pregnancy based on the widely held perception that anticoagulation therapy is likely to benefit both the mother and fetus.

With the identification of the possible role of complement activation in aPL-induced adverse pregnancy outcome, new horizons for novel, safer, and better treatment options are now under investigation (Girardi et al., 2003, 2006; Pierangeli et al., 2005; Xu et al., 2000). Studies in a mouse model have shown that treatment with heparin (UFH or LMWH) blocked complement activation in vivo and in vitro and protected mice from pregnancy complications induced by aPL (Girardi et al., 2004; Salmon and Girardi, 2008). In addition, anticoagulants that do not inhibit complement did not protect pregnancies (Holers et al., 2002). These studies underscore the role of inflammation, rather than thrombosis, in fetal injury associated with aPL. Yet, therapy for pregnant women with APS is currently aimed at preventing thrombosis (Derksen et al., 2004; Levine et al., 2002) and this could explain why anticoagulation is only partially successful in averting complications during pregnancy.

Heparin is usually initiated in the first trimester after the documentation of fetal cardiac activity. The optimal dose of heparin required for safe and effective treatment of women whose APS is diagnosed based on prior fetal loss or neonatal death after delivery prior to 34 weeks of gestation for severe preeclampsia or placental insufficiency is currently being debated. Because these women are at risk of thromboembolic events (Erkan et al., 2001), many experts believe that these cases should be maintained on UFH at doses of 7500–10000 U every 12 hours in the first trimester, to be increased to 10000 U every 12 hours in the second and third trimesters (Branch and Khamashta, 2003). Alternatives include prophylactic doses of LMWH.

4.3. Low-molecular-weight heparin versus unfractionated heparin

The potential complications of heparin treatment during pregnancy include heparin-induced thrombocytopenia, hemorrhage, and osteoporosis. However, osteoporosis and associated bone fractures are rare in pregnant women receiving UFH, reported at a rate of 2.2% (Ruiz-Irastorza et al., 2001b), and even rarer with LMWH (0.5%). Heparin-induced thrombocytopenia, a potentially lethal complication, is also rarely reported in pregnant women (Fausett et al., 2001). LMWH has potential advantages over UFH during pregnancy in terms of less heparin-induced thrombocytopenia (Auger et al., 1995; Warkentin et al., 1995), a lower risk of heparin-induced osteoporosis (Monreal et al., 1994; Nelson-Piercy et al., 1997; Pettila et al., 2002), and a longer plasma half-life with the potential for once-daily administration (Bates et al., 2004), although some authorities believe that twice daily dosing is preferable even when given in prophylactic doses (Petri and Qazi, 2006). When used in therapeutic doses, LMWH does not require laboratory monitoring or dose adjustment in most instances (Hall et al., 1980). At least two small clinical trials have compared UFH versus LWMH in terms of APS pregnancy efficacy. Both showed comparable live birth rates in both groups (Stephenson et al., 2004; Noble et al., 2005). The Cochrane review recommends UFH (Empson et al., 2005) whereas data from two reviews support the use of LMWH as a safer alternative to UFH in pregnancy (Sanson et al., 1999; Walenga et al., 2004).

4.4. Corticosteroids

Although once widely used to prevent aPL-mediated complications in women with APS, several studies (Lockshin et al., 1989; Cowchock et al., 1992; Silver et al., 1993; Laskin et al., 1997) have failed to prove the superiority of a regimen containing corticosteroids over aspirin with or without heparin in women with APS. In fact, corticosteroids increased the rate of complications such as prematurity, diabetes mellitus, and hypertension. Women on long-term corticosteroids should be monitored for the development of maternal complications such as gestational diabetes or hypertension. In addition, corticosteroids can lower the bone mineral density (BMD), and to a greater extent than heparin. Hence, corticosteroids should be reserved for their recommended uses such as in autoimmune thrombocytopenia or coexistent SLE.

4.5. Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG) is of interest because it reduces levels of aCL through saturation of the IgG transport receptor, leading to accelerated catabolism of pathogenic aPL (Pierangeli et al., 2001). Although earlier studies were promising (Spinnato et al., 1995), IVIG is not routinely recommended for pregnant women who have APS. This is based on a lack of clear evidence that it is beneficial. Two small randomized trials (Branch et al., 2000; Triolo et al., 2003) and an observational study (Jeremic et al., 2005) failed to demonstrate any benefit of IVIG in reducing adverse obstetric outcomes in pregnant women who have APS in comparison with the standard combination of heparin and aspirin (Branch et al., 2000; Jeremic et al., 2005; Triolo et al., 2003). The first randomized trial (Triolo et al., 2003) compared IVIG ($n = 21$) with low-dose aspirin and heparin ($n = 19$) in women with recurrent pregnancy loss and aPL. The latter group achieved a higher rate of live births (84%) compared with the IVIG group (57%), without reaching statistical significance (OR 0.25, 95% CI 0.05–1.13). It is worth mentioning that in the pilot study by Branch et al. (2000) that randomized 16 women to receive IVIG or placebo, in addition to heparin/aspirin, IUGR was less common in the IVIG-treated pregnancies, without reaching statistical significance. This suggests that IVIG, with their elevated cost and short supply, should not be used as primary therapy. IVIG may be better reserved for women who are refractory to heparin or prednisone and for those with accepted indications for

IVIG in pregnancy, such as autoimmune thrombocytopenia. This emphasizes the need for large randomized trials and systematic reviews to better evaluate the effectiveness of the use of IVIG for this indication.

4.6. Warfarin

Warfarin should be avoided during pregnancy, especially during the first trimester, because it crosses the placenta and is potentially teratogenic. In some pregnant women protection against arterial thrombosis with therapeutic heparin and low-dose aspirin may not be enough. In those cases, warfarin may be indicated beyond the period of organogenesis (after 12 weeks of gestation) (Hunt et al., 1998), with close monitoring of the international normalized ratio in order to avoid undesirable fetal bleeding. The use of warfarin in the second and third trimesters may be associated with central nervous system abnormalities, such as optic atrophy, mental retardation, delayed development, seizures, microcephaly, and intracerebral hemorrhage (Branch et al., 2000; Stevenson et al., 1980). Pauzner et al. (2001) compared LMWH versus warfarin in 57 pregnancies and found no difference in outcome.

4.7. Plasmapheresis

Plasma exchange is rarely used in women with APS during pregnancy (Frampton et al., 1987; Takeshita et al., 2001) and has not been studied in women with late pregnancy complications. It is postulated that plasmapheresis, through early removal of aPL, might hinder the adverse effects on the trophoblast and therefore reduce the rate of obstetric complications. Ruffatti et al. (2006) reported on their 15-year experience with plasma exchange as a second-line treatment in women with APS and previous thromboembolism who did not respond to conventional treatment. They concluded that prophylactic plasma exchange along with therapeutic anticoagulation and IVIG should be considered in high-risk pregnant APS women. El-Haieg et al. (2007) more recently assessed the impact of plasmapheresis with low-dose prednisone (10 mg/day) on the pregnancy outcome in 18 pregnant women who failed with aspirin and/or heparin. The live birth rate was surprisingly high at 100% with no neonatal deaths related to prematurity and only 11% of cases complicated with IUGR.

Larger randomized controlled trials are needed to better evaluate the role of plasmapheresis in the management of pregnant women with APS.

5. Management algorithm

5.1. Preconception and antepartum

It is well established that women with APS are at risk of late pregnancy loss, hypertensive disorders of pregnancy, prematurity, thrombosis, placental insufficiency, and growth restriction. This emphasizes the need for a multidisciplinary approach towards the management of these women that incorporates a maternal fetal medicine specialist, hematologist/rheumatologist, and neonatologist.

Therefore, the adequate management of women with APS and late pregnancy complications requires preconceptional counseling regarding those potential complications. Each case should be individualized, but women should be informed about an overall live birth rate of 70–80% (Tincani et al., 2003; Lassere and Empson, 2004; Erkan and Lockshin, 2006).

Lupus anticoagulant (LAC) is the best single test for aPL to correlate with the occurrence of thromboembolic events and morbidity during pregnancy (Galli et al., 2003; Opatrny et al., 2006; Urbanus et al., 2008). The diagnostic value of the aCL is currently under debate; however, a recent meta-analysis reports a significant correlation between aCL and both early and late recurrent fetal loss (Opatrny et al., 2006). Evidence is also accumulating that women who test positive for more than one aPL assay have an increased risk of pregnancy morbidity (Ruffatti et al., 2006). Despite that, it remains a matter of debate whether every woman with thrombosis or pregnancy

morbidity should be screened for aCL and other antibody profiles (Urbanus et al., 2008).

It appears that a combination of low-dose aspirin and heparin are the medications of choice for women with obstetric APS in the absence of prior thromboembolic events. However, the optimal regimen is still debatable. In women with APS and one fetal loss, it is recommended to continue aspirin plus heparin, either UFH or LMWH, throughout pregnancy (Petri and Qazi, 2006). A Cochrane Review of 13 studies including 849 women with aPL and a history of miscarriage recommended combination therapy with aspirin and UFH for a 54% reduction in the risk of recurrent miscarriage (Empson et al., 2005). However, they recommended large randomized controlled trials to explore potential differences between UFH and LMWH.

Women receiving heparin should be maintained on calcium, 1000 mg daily, plus vitamin D, 800 IU daily, to lower the frequency of heparin-associated osteoporosis. In the subgroup of women who have preconceptional low BMD, lactation might have to be withheld because of its deleterious effects on bone mass (Ruiz-Irastorza et al., 2001a, 2001 b).

The management approach should also include intense maternal–fetal surveillance throughout pregnancy. The objectives are close observation of maternal blood pressure and other features of preeclampsia. These include checking for proteins in the urine every visit, frequent prenatal visits every two weeks before midgestation and every week thereafter, and periodic obstetric ultrasounds to assess fetal growth and amniotic fluid volume, and appropriate fetal surveillance testing (Branch and Khamashta, 2003). This should probably be instituted as early as 32 weeks of gestation, or even earlier if growth restriction or other features of placental insufficiency such as oligohydramnios are suspected and should be continued at least weekly until delivery. Particularly in women with secondary APS in the presence of underlying autoimmune disease such as SLE, the presence of risk factors such as proteinuria, thrombocytopenia, and hypertension, especially in the first trimester, increases the risk of pregnancy loss (Clowse et al., 2006b). Uterine and umbilical artery Doppler flow studies performed at 20–24 weeks of gestation might be helpful in predicting the development of preeclampsia and placental insufficiency in women with APS (Le Thi Huong et al., 2005, 2006; Papageorghiou and Roberts, 2005). Le Thi Huong et al. found that 72.2% of pregnancies with abnormal second trimester Doppler studies would be complicated with preeclampsia, prematurity, fetal death, and/or IUGR. Particularly in women with SLE, periodic rheumatologic consultation every 3–4 weeks is advisable throughout pregnancy.

5.2. Intrapartum management

Thromboprophylaxis may pose a risk intrapartum, especially in women requiring epidural anesthesia. Therefore, to reduce the risk of bleeding at the time of delivery an adequate management plan would be to electively induce labor at 37 weeks of gestation and at least 12 hours after discontinuing prophylactic LMWH (Ruiz-Irastorza and Khamashta, 2007). Variations on these recommendations include the use of therapeutic UFH after 37 weeks, allowing spontaneous labor. Aspirin can be continued until delivery. The use of low-dose aspirin should not affect the use of regional anesthesia intrapartum since there is no evidence that it increases the risk of epidural hemorrhage (Shehata et al., 2001). However, many anesthesiologists require a minimum of 3–7 days without aspirin before performing regional anesthesia (Wetzl, 2004). In women who develop HELLP syndrome, prompt delivery is recommended and plasma exchange sessions might be required to ameliorate the symptoms in some unresponsive cases (Kupferminc et al., 1994).

5.3. Postpartum management

Thromboprophylaxis in the postpartum period in women with APS and prior thrombosis is critical because this is a period with a high risk of recurrent thrombosis (Ginsberg et al., 2001). This can be easily accomplished by reinstitution of LMWH and shifting to warfarin, which is safe for

breastfeeding mothers, in women who were on oral anticoagulation preconceptionally as soon as hemostasis is achieved. In most cases, the aim is to achieve an international normalized ratio of 3.0 (Branch and Khamashta, 2003). However, the postpartum management of women without prior thrombosis in whom APS is diagnosed because of prior fetal loss or neonatal death after delivery, or diagnosed secondary to severe preeclampsia or placental insufficiency, is less certain. Most experts recommend postpartum LMWH for all women with obstetric APS and it is also recommended in asymptomatic women with aPL, especially those with SLE (Ruiz-Irastorza and Khamashta, 2005). The duration of treatment varies from 3–5 days in the United Kingdom, especially in the event of cesarean delivery, to 6–8 weeks in the United States (Branch and Khamashta, 2003).

6. Management of specific complications

6.1. Catastrophic antiphospholipid syndrome

Catastrophic APS is a rare, life-threatening variant of APS that is characterized by multiple organ failure secondary to microangiopathic thrombosis (Asherson, 1992). Catastrophic APS in pregnancy represents around 6% of all described cases and is associated with a high maternal mortality rate (Gomez-Puerta et al., 2007). It is characterized by a spectrum of clinical and hematological features including central nervous system involvement, HELLP syndrome, disseminated intravascular coagulopathy (Asherson et al., 2005), and small-vessel thrombosis. Management of catastrophic APS in pregnancy should aim at preventing any trigger factors, such as infection, and maintenance of adequate anticoagulation. In the presence of HELLP syndrome and other microangiopathic features, plasma exchange is indicated (Gomez-Puerta et al., 2007) and has been proven to improve survival in women with catastrophic APS (Ruffatti et al., 1994; Vora et al., 2006). Steroids and IVIG can be used, but most importantly; immediate delivery should be achieved to save the mother and the fetus.

6.2. Refractory cases

In women with APS, adverse pregnancy outcome can still occur in 20–30% of cases despite treatment with heparin, with or without aspirin. A properly designed trial evaluating treatment of ''refractory'' APS has never been conducted. Despite that, clinicians have tried alternative therapy in a subsequent pregnancy. In women whose treated pregnancy failure occurred on a prophylactic-dose regimen, full-dose anticoagulation in the next pregnancy seems rational. Corticosteroids, often in substantial doses, have also been tried. By the mid-1990 s, IVIG in conjunction with heparin and low-dose aspirin, was also attempted in selected cases. In summary, if pregnancy failure occurs despite full anticoagulation, most authorities would be inclined to add an immunomodulatory agent such as corticosteroids or IVIG to the anticoagulation regimen.

6.3. Patients with systemic lupus erythematosus

APS is a predictor of adverse pregnancy complications in pregnant women with SLE, most notably predicting a high risk of second and third trimester losses and stillbirths (Yasmeen et al., 2001; Simpson, 2002). In women with active SLE who are maintained on hydroxychloroquine, there is a consensus to continue treatment during pregnancy (Witter, 2007). Although hydroxychloroquine crosses the placenta, it has been used safely in pregnancy with no reported fetal toxicity (Levy et al., 2001; Clowse et al., 2006a). The management of these pregnancies is otherwise similar to APS in the absence of SLE. Since preeclampsia and growth restriction are more common in women with SLE, close antenatal follow-up is further stressed in those women. In the presence of chronic hypertension, control of blood pressure is required to decrease maternal morbidity.

7. Conclusions

Although routine use of prophylactic-dose UFH or LMWH plus low-dose aspirin is generally advocated for pregnant women with APS and adverse obstetric outcome, this approach is supported by questionable data since these women are underrepresented in clinical trials. Corticosteroids and IVIG are not recommended as first-line treatment for pregnant women with APS. Warfarin is generally contraindicated, and its use should be limited to special conditions. Since most treatment regimens proposed for women with APS carry a significant risk for both the mother and the fetus, each woman should be extensively counseled, and therapeutic options should be individually tailored based on her individual risk of aPL-mediated complications.

Complement activation has been proposed as a novel mechanism to explain aPL-associated obstetric complications in women with APS. If this proves true in human trials, it will open new horizons for interventional trials to evaluate the role of complement inhibitors in preventing aPL-associated fetal loss and adverse pregnancy outcome.

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CHAPTER 18

Difficult Clinical Situations in the Antiphospholipid Syndrome

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1. Introduction

Despite the increased understanding of the mechanisms and clinical manifestations of the antiphospholipid syndrome (APS), very few studies address the treatment of antiphospholipid antibody (aPL)-positive patients in a risk-stratified, prospective, and controlled manner. Furthermore, the literature on the non-criteria features of aPL such as thrombocytopenia or cardiac valve disease is limited. Thus, the management of some aPL-positive patients remains largely empirical, and, in daily practice, physicians are frequently faced with challenging APS questions without evidence-based answers.

The purpose of this chapter is to review the management of the difficult clinical situations in APS and to provide the reader with information that may aid in their decision-making process. The previous chapter provides a comprehensive approach to primary and secondary thrombosis prevention in aPL-positive patients as well as the management of pregnancies. Difficult clinical situations in APS addressed here (Table 1) are chosen because of existing controversies in patient management and/or the lack of published data.

2. Catastrophic antiphospholipid syndrome

Catastrophic APS (CAPS) is a rapidly progressive life-threatening form of APS that causes multiple organ thromboses, generally associated with microvascular involvement and microangiopathic conditions (Asherson et al., 2003). High index of clinical suspicion and careful investigation are required to make an early diagnosis so that aggressive treatment can be initiated. Despite multimodal treatment, CAPS is still associated with high mortality; evidence-based management recommendations do not exist due to the rarity of the condition and the lack of controlled studies. Thus, current treatment recommendations are based on the reported case series and the descriptive analysis of the International Web-based CAPS Registry (Asherson et al., 2003); patients who receive the combination of anticoagulation plus corticosteroids plus intravenous immunoglobulin (IVIG) or plasma exchange have the best survival rates (Erkan et al., 2003a).

The justification for the use of the above agents comes from the observations that:

- Heparin inhibits clot formation, lyses existing clots, inhibits the binding of aPL to their target in ELISA (Franklin and Kutteh, 2003), and prevents aPL-induced complement activation in animal APS models (Girardi et al., 2004).
- Corticosteroids inhibit systemic inflammatory response syndrome-related excessive cytokine response (Scheinman et al., 1995) via suppression of the humoral and cell-mediated immune responses; moreover aPL induce

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Table 1
Difficult clinical situations in antiphospholipid syndrome

1. Catastrophic APS
2. Recurrent events despite optimal anticoagulation
3. The optimal duration of anticoagulation
4. Thrombocytopenia
5. Nephropathy
6. Cardiac valvular disease
7. Cognitive dysfunction independent of stroke
8. Non-healing skin ulcers
9. Lupus-anticoagulant hypoprothrombinemia syndrome (LAC-HPS)
10. Diffuse alveolar hemorrhage
11. Dysfunctional uterine bleeding
12. Perioperative medical management of APS patients
13. Endovascular procedures in APS patients

procoagulation and proinflammation via the nuclear factor (NF)-κB pathway (Dunoyer-Geindre et al., 2002) and corticosteroids inhibit NF-κB activation (Raschi et al., 2003).

- IVIG blocks autoantibodies, increases clearance of pathologic IgG, modulates complement, protects against autoantibody-mediated pathology by upregulating an inhibitory Fcγ receptor on macrophages (Nimmerjahn and Ravetch, 2007), and suppresses pathogenic cytokines (Knezevic-Maramica and Kruskall, 2003).
- Plasma exchange has been successfully used in CAPS patients (Flamholz et al., 1999) and is thought to be effective due to removal of autoantibodies.

In the case of a deteriorating clinical situation, an additional agent such as cyclophosphamide or rituximab is generally used. Based on a recent analysis of the web-based CAPS registry, systemic lupus erythematosus (SLE) is a poor prognostic factor in CAPS patients and cyclophosphamide may be beneficial in SLE–CAPS patients but not in primary CAPS patients; these results can be confounded by the fact that the timing of cyclophosphamide was earlier in the course of SLE–CAPS management than in primary CAPS (Bayraktar et al., 2007a). Similarly, rituximab (an anti-CD20 monoclonal antibody) has been successfully used in CAPS patients with thrombocytopenia or autoimmune hemolytic anemia (Erdozain et al., 2004; Tommasino et al., 2004; Yamazaki et al., 2004, Rubenstein et al., 2006); however, it is not possible to evaluate the antithrombotic effects of rituximab since patients in the published reports received anticoagulation in addition to multiple immunosuppressive agents (see the RITAPS clinical trial in Section 5.1 for further discussion).

Given that inhibiting complement activation prevents experimental aPL-induced fetal death, C5 knockout mice carry pregnancies normally despite aPL (Girardi et al., 2006), and complement activation is required for experimental thrombosis (Fleming et al., 2004), complement inhibition may have a future role in the management of CAPS patients.

Several other points discussed below are critical in CAPS patients:

- Thrombosis with IVIG use has been reported when high doses are delivered rapidly, especially in elderly patients with other co-morbidities such as diabetes, hypertension, or hypercholesterolemia (Orbach et al., 2005). When anticoagulation needs to be interrupted in CAPS patients, physicians should be cautious with IVIG infusions. Avoiding other products with high osmolality, reducing the rate of IVIG infusion to avoid large osmolar load delivery over short periods, hydration, and using non-sucrose IVIG products (especially in patients with renal failure) are some of the strategies that can reduce the risk of IVIG-related thrombosis (Chapman et al., 2004; Fleming et al., 2004).
- Although plasma exchange improves outcomes in CAPS patients, it is important to note that most, but not all, of reported CAPS patients received plasma exchange together with fresh frozen plasma as the replacement fluid. Fresh frozen plasma contains natural anticoagulants (such as antithrombin III and protein C) as well as clotting factors. It is unknown if plasma exchange with fresh frozen plasma or with a different replacement fluid such as human albumin solution would result in different outcomes in CAPS patients. There are two CAPS patients who successfully received

plasma exchange with albumin solution and antithrombin III concentrate replacement at the end of each session; the true effectiveness of this approach cannot be assessed in these patients due to other confounders (Marson et al., 2008).

- When IVIG and plasma exchange are used simultaneously in the same patient, usually IVIG is administered 48–72 hours after the plasma exchange in order to prevent the removal of IVIG by plasma exchange (IVIG has rapid distribution to the extracellular space within 48 hours).
- Overlap between CAPS and other microangiopathic conditions, such as thrombocytopenic purpura, may exist; in fact, CAPS patients with thrombocytopenia, compared with those without thrombocytopenia, have higher incidence of concomitant hematological manifestations (hemolysis, schistocytes, disseminated intravascular coagulation, and elevated fibrin degradation products) (Bayraktar et al., 2007b). Thus, the term "microangiopathic aPL-associated APS" was suggested for this group of patients (Asherson and Cervera, 2008).
- Adrenal failure due to infarction/hemorrhage can occur in 10–26% of CAPS patients (Espinosa et al., 2003) who may present with abdominal or back pain followed by rapid vascular collapse. During critical illness, one should recognize the corticosteroid insufficiency when peak cortisol concentrations are $<20\,\mu g/dL$ at all time points of an acute adrenocorticotropic hormone test or when cortisol increment is $<9\,\mu g/dL$ (Annane, 2003). These patients, if not receiving high-dose corticosteroids, should be treated with intravenous bolus of 50 mg of hydrocortisone every 6 hours combined with 50 μg of fludrocortisone given orally once a day for 7 days. Because critical illness-induced corticosteroid insufficiency is usually transient, long-term replacement therapy is not mandatory, but permanent insufficiency due to adrenal infarction/hemorrhage should be excluded in patients that are critically ill with CAPS (Vero et al., 2006).
- When bleeding occurs in CAPS patients, the timing of anticoagulation is a difficult decision that physicians must make in APS patients. Anticoagulation should be started as soon as bleeding is controlled; knowing that the risk of further bleeding remains high (Silverberg et al., 2002).
- Catastrophic APS recurrence is unusual and patients generally have a stable course with continued anticoagulation. Almost two-thirds of patients who survive an initial catastrophic APS event remain symptom free with anticoagulation in an average follow-up of 67 months (Erkan et al., 2003b).

3. Recurrent events despite optimal anticoagulation

The best approach in APS patients who have recurrent thrombotic events despite optimal anticoagulation with warfarin is unknown. No large-scale study has addressed the treatment options for these patients. Multiple factors may contribute to the failure of conventional therapy, most importantly persistent non-aPL thrombosis risk factors. Treatment failures may also be due in part to warfarin resistance in APS patients, which may be caused by an unknown acquired or genetic trait (Valesini and Pittoni, 2000; Schwarz et al., 2008). Drug interactions are another reason for warfarin resistance; for example azathioprine interacts with warfarin and reduces its efficacy by possible hepatic enzyme induction (Rivier et al., 1993). Thus patients who receive warfarin and azathioprine concomitantly should be assessed carefully. Other medications may have similar effects.

In a recent editorial, Kasthuri and Roubey emphasized the point that factor II (prothrombin) level is the most important indicator of warfarin effectiveness as it more accurately reflects thrombin generation; however, the International Normalized Ratio (INR) level is most dependent on factor VII levels and least on factor II (Kasthuri and Roubey, 2007). Thus, given that the INR may not be the best measure of warfarin effectiveness, factor II assay can be considered in patients with recurrent events despite optimal anticoagulation (Kasthuri and Roubey, 2007).

Identification and possible reduction of additional thrombotic risk factors is crucial in APS patients with recurrent thrombosis, as synergistic actions between aPL and other clinical risk factors have been well recognized (Erkan et al., 2002a). Patients should be advised to stop smoking, have well-controlled blood pressure and sugar, and women should be counseled against the use of estrogen-containing oral contraceptive pills or hormone replacement therapy. Patients should introduce lifestyle changes to achieve and maintain ideal weight, cholesterol level, and physical activity. Other forms of hereditary hypercoagulable states such as prothrombin 20210 mutation, factor V Leiden mutation, or hyperhomocysteinemia should be considered in all aPL-positive patients, especially in those with recurrent thrombosis, as coexistent conditions particularly increase the risk of thrombosis (Schutt et al., 2000; Seriolo et al., 2001; Erkan et al., 2007). Elevated homocysteine levels can be reduced to normal with vitamin B6 or folic acid.

In recurrent thrombosis despite optimal warfarin treatment (INR: 2–3), the options include: adding low-dose aspirin (50–325 mg daily), hydroxychloroquine (HCQ), and/or statin; (b) switching to low-molecular-weight heparin, or (c) increasing the warfarin dose to achieve a higher INR (INR to 3–4) with or without low-dose aspirin, HCQ, or statin.

Whether the addition of low-dose aspirin in this situation is effective is unknown, but it can be considered in patients especially with arterial thrombosis. However, it is clear that the risk of bleeding is increased with combination therapy (Johnson et al., 2008).

Hydroxychloroquine has been used as a prophylactic agent against deep venous thrombosis (DVT) in hip surgery patients (Petri, 1998). Furthermore, it reduces the risk of arterial thrombosis in both SLE patients and animal models for APS (Edwards et al., 1997; Petri, 1998), and decreases the anticardiolipin antibody (aCL) titers in some studies (McCarty and Cason, 2004) but not in all (Erkan et al., 2005). In addition to anti-inflammatory effects, it possesses an antithrombotic effect by inhibiting platelet aggregation (Jancinova et al., 1994) and arachidonic acid release from stimulated platelets (Yoon, 2002). Other potential immunomodulatory effects of HCQ include increasing the pH of intracellular vacuoles and interfering with antigen processing (Lombard-Platlet et al., 1993), and inhibiting T-cell receptor- and B-cell antigen receptor-induced calcium signaling (Goldman et al., 2000). In aPL-injected mice, HCQ decreases the thrombus size and the time of thrombus in a dose-dependent manner (Edwards et al., 1997). Furthermore, HCQ inhibits the aPL-induced glycoprotein IIb/IIIa receptor expression in a dose-dependent fashion (Pierangeli et al., 2004). Even though there are not enough data to recommend HCQ as the only treatment for secondary prevention of thrombosis, it may be reasonable to add HCQ to anticoagulation in APS patients who develop recurrent thrombosis despite optimal anticoagulation.

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are used widely for the treatment of hypercholesterolemia. Clinical trials in patients with and without coronary heart disease and with and without high cholesterol have demonstrated consistently that statins reduce the relative risk of major coronary events by 30% (Maron et al., 2000). Statins also have anti-inflammatory roles, including decreasing the expression of adhesion molecules in monocytes, interfering with leukocyte–endothelial interaction, inhibiting platelet function, and downregulating inflammatory cytokines in endothelial cells (Pierangeli et al., 2004). Furthermore, endothelial activation mediated by anti-β_2-glycoprotein I antibody can be inhibited by statins (Meroni et al., 2001). Whether statins are effective in the setting of recurrent thrombosis is unknown, however they can be considered an addition to anticoagulation when patients continue to clot despite optimal therapy.

Another approach is to maintain the patient on chronic heparin therapy. Low-molecular-weight heparin (LMWH) is generally preferable to unfractionated heparin; the advantages of LMWH include the lower frequencies of bleeding complications, heparin-induced thrombocytopenia, and heparin-induced osteoporosis. Based on our retrospective experience with eight patients, there was no recurrence in three patients who were

warfarin-naïve (never received warfarin) after 7 patient years, and there was one recurrence in five patients who were warfarin-exposed (initially received warfarin) after 25 patient years (Kaul et al., 2005). However, controlled studies of LMWH have not yet been carried out.

New antiplatelet agents such as dipyridamole, ticlopidine, or clopidogrel (Plavix) as well as oral factor II and X inhibitors under development will likely have a more important role in the management of APS patients in the future, but have not been formally studied at this time. We have retrospectively demonstrated that out of eight warfarin-naïve APS patients who had been treated with antiplatelet agents (six patients with clopidogrel, one with dipyridamole, and one with aspirin with dipyridamole), only one had recurrent stroke while on clopidogrel after a total of 17 patient years (Kaul et al., 2005).

For patients with recurrent thrombosis, in addition to medical management, inferior vena cava filter placement can be considered in order to prevent pulmonary embolus (see Section 14 for further discussion). Immunosuppression can be considered for selected APS patients, even in the absence of CAPS, but the decision should be individualized.

In summary, the first step in APS patients with recurrent thrombotic events despite optimal anticoagulation with warfarin is the identification and possible reduction of non-aPL thrombosis risk factors. As a second step: (a) adding low-dose aspirin, HCQ, and/or statin; (b) switching to LMWH, or (c) increasing the warfarin dose to achieve a higher INR (INR 3–4) with or without low-dose aspirin, HCQ, or statin can be considered.

4. Long-term management of anticoagulation in APS patients

There is no evidence that the anticoagulation should be indefinite in APS patients who develop events in the presence of other clinical thrombotic risk factors or a precipitating event. In addition, the optimal duration of anticoagulation in stable APS patients without other risk factors is unknown.

Retrospective uncontrolled studies show APS patients have a high a risk of recurrent thrombosis after discontinuation of anticoagulation therapy (Rosove and Brewer, 1992; Derksen et al., 1993; Khamashta et al., 1995). However, these studies are based on relatively small numbers of patients, are subject to selection and referral biases, and have not included an extensive evaluation of risk factors other than aPL. Two prospective studies confirmed that aPL are associated with a twofold or greater risk of recurrent thrombosis after cessation of anticoagulation (Schulman, et al., 1998; Kearon et al., 1999). Schulman and colleagues demonstrated that the risk of a recurrent venous event is 29% over 4 years in aCL-positive patients, compared with 14% in aCL-negative patients (relative risk (RR) 2.1, 95% confidence interval (CI) 1.3–3.3) (Schulman et al., 1998). However, these prospective studies also did not address the non-aPL thrombosis risk factors, the optimal duration of therapy, or when anticoagulation can be discontinued.

Well-established risk factors for thrombosis such as hypertension, smoking, hyperlipidemia, postoperative state, immobilization, peripartum period, or oral contraceptive use can coexist in APS patients at the time of an event, and may even be responsible for triggering acute thrombosis (Seriolo et al., 2001; Erkan et al., 2002a; Rubenstein et al., 2006). Based on our experience, at least 50% of APS patients with vascular event have other non-aPL thrombosis risk factors at the time of thrombosis (Erkan et al., 2002a; Kaul et al., 2007). Thus, whether APS patients should receive lifelong anticoagulation after an event, which occurs in the setting of a high-risk situation, or after years of stable disease is debatable. The decision to stop anticoagulation is challenging. Severity and the location of thrombosis, aPL profile (low-titer aCL versus high-titer aCL and/or positive lupus anticoagulant test), whether residual thrombosis or atherosclerosis exists, the coexistence of other thrombotic clinical risk factors at the time of the event, and patient characteristics including age, lifestyle, and comorbid conditions should be factored into one's clinical equation. Location of the thrombosis deserves special attention because APS patients tend to have a subsequent event that

is similar to the initial event (arterial event followed by arterial, and venous event followed by venous) although the reason is unknown. Thus, physicians may be more comfortable stopping warfarin in patients with history of venous thrombosis (especially if the event had occurred in the presence of other clinical risk factors) compared with patients with history of arterial thrombosis.

In summary, currently, "long-term" anticoagulation with warfarin is the recommended therapy for APS patients with vascular events. However, if there is no recurrence in the long-term follow-up, one can consider that switching to a safer antiplatelet agent such as aspirin may be appropriate. There is an absolute need for large-scale prospective studies in which patients are stratified for other underlying clinical risk factors. Until these studies are available, the risks and benefits of stopping warfarin treatment should be discussed with patients very carefully.

5. Thrombocytopenia

About 20–30% of patients with APS have thrombocytopenia. Although the mechanism is not clear, there is some evidence that aPL bind to platelet membranes and cause platelet destruction (Trappe et al., 2006). In most cases, the thrombocytopenia is mild (>70000 platelets per microliter) (Cuadrado et al., 1997) and not associated with major bleeding (Galli and Barbui, 2006). Thus, thrombocytopenia in aPL-positive patients rarely requires treatment.

When severe thrombocytopenia exists in aPL-positive patients, the first-line therapeutic options include high-dose corticosteroids and/or IVIG. Rituximab has recently been used successfully to treat thrombocytopenia in aPL-positive patients (Table 2) (see below for further discussion). As a last resort, splenectomy is an option, however this should be considered with extreme caution because of the increased risk of both bleeding and clotting in the setting of surgery in aPL-positive patients (Galli and Barbui, 2006). One difficult clinical dilemma is APS patients developing thrombosis with severe thrombocytopenia (<30000 per microliter). Not addressing the clot may be life-threatening, while anticoagulation can cause a similarly threatening bleeding; starting low-dose anticoagulation in addition to the immunosuppressive treatment while monitoring closely for bleeding can be considered.

In summary, although most of the aPL-positive patients with thrombocytopenia do not require treatment, corticosteroids, IVIG, rituximab, or splenectomy can be considered when clinically significant thrombocytopenia occurs.

5.1. RITAPS pilot trial

Our descriptive retrospective study of five patients provided evidence that B cell depletion with rituximab is well tolerated and effective for refractory thrombocytopenia (and skin ulcers) in aPL-positive patients (Tenedios et al., 2005a). Based on this experience, and also given the in vitro experience that B cells are involved in aPL-related clinical events (Akkerman et al., 2004; Youinou and Renaudineau, 2004), we designed the RITAPS trial, an open-label phase IIa descriptive pilot study in which the effectiveness and safety of rituximab was assessed in 20 patients with anticoagulation-resistant manifestations of aPL. The inclusion criteria of the RITAPS trial include (in addition to persistent aPL-positivity): persistent thrombocytopenia; persistent autoimmune hemolytic anemia; cardiac valve disease; chronic skin ulcers; renal thrombotic microangiopathy; and/or cognitive dysfunction (confirmed by neuropsychological batteries with/without white matter changes). We believe that the RITAPS trial will provide systematic data on the clinical, laboratory (including thrombocytopenia), and serologic parameters of rituximab-receiving aPL-positive patients (www.clinicaltrials.gov/ct2/show/NCT00537290?term=erkan&rank=1).

6. Nephropathy

The renal manifestations of aPL include thrombosis in the renal artery trunk or its branches,

Table 2
Rituximab for the treatment of resistant thrombocytopenia in antiphospholipid syndrome

Demographics	History	Prior treatment	Indication for rituximab	Reference
32-year-old man	Thrombocytopenia DVT, PE Renal failure Mitral insufficiency Skin ulcers	IVIG Corticosteroids Warfarin	Need for long-term anticoagulation in the setting of thrombocytopenia	Trappe et al. (2006)
30-year-old woman	DVT, PE, Budd–Chiari, CVA, chronic ITP, subdural hematoma on warfarin	Plasmapheresis, cyclophosphamide Warfarin	Life-threatening thrombosis in the setting of thrombocytopenia	Ahn and Lander (2005)
34-year-old man	ITP Multiple thrombi	Corticosteroids Splenectomy IVIG Plasmapheresis Cyclophosphamide Vincristine Azathioprine Warfarin	Life-threatening thrombosis in the setting of thrombocytopenia	Rubenstein et al. (2006)
54-year-old man	Thrombocytopenia Myocardial infarction	Corticosteroids Vincristine Danasol IVIG Cyclophosphamide	Thrombocytopenia	Rubenstein et al. (2006)
27-year-old woman	Thrombocytopenia Multiple thrombi Budd–Chiari syndrome	Corticosteroids Heparin	CAPS and thrombocytopenia	Rubenstein et al. (2006)
39-year-old woman	Thrombocytopenia Multiple thrombi Skin ulcers Ectopic pregnancy Miscarriage Mural thrombus	Corticosteroids Warfarin Hydroxychloroquine Danazol IVIG Azathioprine Cyclosporine Dapsone Cyclophosphamide Vincristine	Life-threatening thrombosis in the setting of thrombocytopenia	Anadacoomarasamy et al. (2006)

Note: All doses of rituximab were 375 mg/m^2 weekly × 4 weeks.
All patients had improved thrombocytopenia and no further thrombotic events.
PE, pulmonary embolism; DVT, deep venous thrombosis; CVA, cerebrovascular accident; ITP, immune thrombocytopenic purpura; IVIG, immune globulin; CAPS, catastrophic antiphospholipid syndrome.

intraparenchymal arteries and arterioles, glomerular capillaries, and the renal veins (Uthman and Khamashta, 2006). The most common and characteristic intrarenal vascular lesion in patients with primary APS is thrombotic microangiopathy (TMA), which is histologically defined as focal or diffuse microangiopathic changes affecting the intrarenal vascular tree and the glomerular tufts with acute and chronic recanalizing thrombi (Amigo, 2006). Patients with TMA can present with hypertension, proteinuria (mild to nephritic range), hematuria, and/or slowly progressive renal failure (Uthman and Khamashta, 2006). When other systemic symptoms exist it can be difficult to distinguish aPL-positive patients with TMA from other microangiopathic conditions such as thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) (Amigo, 2006).

Clinicians should persistently monitor aPL-positive patients for any signs of renal disease. Antiphospholipid antibody-related nephropathy is generally diagnosed by kidney biopsy, although Doppler ultrasonography examination of the intrarenal distal arteries may be another diagnostic

approach in the future (Kirsanova et al., 2008). There is no clear consensus on the management of nephropathy in aPL-positive patients and it depends to a large extent on the patient's clinical manifestations. Based on a limited number of case reports: (a) one patient had disappearance of proteinuria and normalization of creatinine clearance over a two-month period when treated with angiotensin-converting enzyme (ACE) inhibitor and aspirin (Hamidou et al., 1995); (b) Korkmaz et al. reported improvement in proteinuria and renal function in a series of patients on a cocktail of immunosuppressives, anticoagulation, calcium channel blocker, and an ACE inhibitor (Korkmaz et al., 2003); and (c) there are reports of the use of plasmapheresis or corticosteroids with varying success (Hughson et al., 1992; Korkmaz et al., 2003).

In summary, aPL nephropathy is usually slowly progressive with no proven treatment. Although most of the patients are treated with ACE inhibitors and anticoagulation, the effectiveness of this approach is not known and a better understanding of the pathophysiology of aPL nephropathy will be beneficial in guiding treatment. Of note, one of the inclusion criteria of the ongoing RITAPS trial (see Section 5.1 for further discussion) is biopsy-proven aPL nephropathy, which will help determine the role of an immunosuppressive approach in these patients.

7. Cardiac valvular disease

Heart valve abnormalities (vegetations and/or thickening) are the most common cardiac manifestation in APS patients, with the mitral valve being the most likely affected, followed by the aortic valve (Tenedios et al., 2005 b). A third to half of patients with primary APS have valvular disease and it is usually asymptomatic (Long and Leya, 2008); however, about 5% will progress to cardiac failure and require valve replacement (Tenedios et al., 2005 b). In addition, 48% of aPL-positive SLE patients have cardiac valve disease (Cervera, 2005).

A proposed mechanism for the formation of the valvular lesion is aPL-mediated endothelial activation with prominent deposition of aPL in heart valves, initiating an inflammatory process mediated by complement (Tenedios et al., 2005 b). On histopathology, these lesions are characterized by superficial and intravalvular deposits of fibrin with subsequent organization (Espinola-Zavaleta et al., 1999).

The optimal treatment of aPL-related cardiac valve disease in the absence of clot is unknown. Arterial thrombosis occurs more commonly in primary APS patients with valvular lesions (Espinola-Zavaleta et al., 1999). Because of the increased risk of systemic embolization from valve thickening and vegetations (Bulckaen et al., 2003), anticoagulation is an option; although there are anecdotal reports of the resolution of mitral valve vegetations with high-intensity warfarin (Skyrme-Jones et al., 1995; Agirbasli et al., 1997), no controlled data exist demonstrating the resolution or improvement of valve lesions with anticoagulation. Espinola-Zavaleta performed transesophageal echocardiograms (TEE) in 12 primary APS patients with a 5-year follow-up and found that oral anticoagulant treatment (acenocoumarin) with an INR goal of 2.5–3 and aspirin (100 mg/day) was not effective in the regression of valvular lesions (70% of the patients had valvular lesions on the first TEE, while 100% had lesions at the 5-year follow-up) (Espinola-Zavaleta et al., 2004). The role of antiplatelet agents is also unclear but aPL-positive patients with cardiac valve disease are usually treated with low-dose aspirin.

The administration of corticosteroids does not appear to have a significant effect on valvular morphology (Morita et al., 2000). Corticosteroids may be considered in APS-related hemodynamically unstable valvular abnormalities, especially when symptoms are of recent onset (Nesher et al., 1997). It has been suggested, however, that corticosteroids may facilitate healing of valvular vegetations, which paradoxically may result in marked scarring and deformity of the valve that results in valve dysfunction (Hojnik et al., 1996).

As with other valvulopathies, patients with aPL-associated valve abnormalities may require prophylaxis with antibiotics before invasive procedures.

In summary, there are no studies demonstrating the benefit of antiplatelet agents, anticoagulation, or immunosuppressive agents in aPL-related cardiac valve disease. Anticoagulation can be considered in high-risk patients such as those with atrial fibrillation or multiple non-aPL thrombosis risk factors.

8. Cognitive dysfunction independent of stroke

Cognitive dysfunction in primary APS (without other systemic autoimmune diseases) or in lupus-associated APS can be seen independent of stroke. However, relatively little is known about the cognitive pattern in these two selected groups, and very little information is available regarding the significance and management of white matter changes that may have an association with the underlying attention and executive cognitive impairment. Studies that have looked at cognition in primary APS or in asymptomatic aPL-positive patients have shown that cognitive deficits may be present independent of any history of known CNS involvement (Jacobson et al., 1999; Tektonidou et al., 2006). These patients may complain of difficulty with memory, attention, and concentration, or the dysfunction may be subclinical and apparent only with neuropsychological testing.

It is likely that cognitive dysfunction in these patients is due to multiple mechanisms. The underlying pathophysiology may relate to small vessel ischemic events, or there may be a direct pathogenic role of aPL, which has implications in the treatment. Antiphospholipid antibodies may bind to the cells of the CNS (Caronti et al., 1998) followed by permeabilization and depolarization of these cells (Chapman et al., 1999). Animal models corroborate that neuropsychiatric performance is affected by aPL independently of the ischemic events (Ziporen et al., 1997; Katzav et al., 2001; Shoenfeld et al., 2003). In humans, an association has been demonstrated between cognitive dysfunction and both livedo reticularis and white matter lesions on magnetic resonance imaging (MRI) (Tektonidou et al., 2006). This finding is suggestive of a microangiopathic mechanism.

With limited understanding of pathogenesis, the optimal treatment strategy for isolated aPL-associated cognitive dysfunction is also yet to be defined. The efficacy of any particular treatment, antithrombotic or immunosuppressive, has not been demonstrated clearly. There have been anecdotal reports suggesting a benefit for anticoagulation for cognitive dysfunction, but this has never been corroborated prospectively (Hughes et al., 2001). More benign therapy with antiplatelet or antimalarial agents may also be justified, although there are no clinical data supporting their effectiveness. The effectiveness of an immunosuppressive approach is also unknown; given that aPL–CNS interaction is one of the potential mechanisms of aPL-induced cognitive dysfunction, one of the inclusion criteria of the ongoing RITAPS trial (see Section 5.1 for further discussion) is neuropsychological test-proven cognitive problems.

A different approach to this problem is the consideration of cognitive rehabilitation, if available. In a small, uncontrolled pilot study of 17 high-functioning SLE patients (not all of whom were aPL-positive) with cognitive complaints but normal neuropsychiatric testing, an 8-week cognitive intervention program showed improvement in meta-memory (the knowledge one has about memories) and memory self-efficacy scores as well as improvement in the Beck Depression Inventory. The intervention was weekly 2-hour psychoeducational group sessions teaching cognitive strategies and giving psychosocial support (Harrison et al., 2005). A commonsense approach to addressing the problem of cognitive dysfunction in an aPL-positive patient would also include addressing any comorbid conditions that can affect cognitive function, from other medical disease to medication side-effects (Harrison and Ravdin, 2006).

Given the fact that understanding the true prevalence and mechanisms of cognitive impairment in aPL-negative SLE and aPL-positive non-SLE patients will facilitate early diagnosis and eventual treatment options, a pilot neuropsychological and functional MRI study designed to provide information for larger studies of SLE and APS populations regarding the prevalence and mechanism of cognitive dysfunction is underway

(www.hss.edu/understanding-clinical-trials_sle-apl-mri-cognitive-dysfunction.asp).

In summary, there is no proven therapy for aPL-positive patients with cognitive dysfunction; both mechanistic and therapeutic studies (RITAPS trial—see Section 5.1 for further discussion) are underway.

9. Non-healing skin ulcers

After livedo reticularis, non-vasculitic skin ulcers, including widespread cutaneous necrosis, are the second most common skin manifestation of aPL (Frances et al., 2005; Asherson et al. 2006). Widespread cutaneous necrosis due to thrombosis of the microvasculature can be a therapeutic dilemma, and multiple different approaches have been used. Low-dose aspirin and/or dipyridamole have been suggested as the first approach to APS-related small ulcers in addition to local care (Nahass, 1997). More aggressive therapy with oral anticoagulation and/or antifibrinolytic therapy has also been demonstrated to be effective in some APS patients with recurrent ulcerations (Alegre et al., 1989; Gertner and Lie, 1994; Nahass, 1997; Aguirre et al., 1998); however this approach is not always effective in our experience.

In patients with skin ulcers recalcitrant to anticoagulation and antiplatelet therapy, case reports have shown success with intravenous recombinant tissue plasminogen activator (rTPA) (Srinivasan et al., 2001), sildenafil (Gertner, 2003), and 5-aminolevulinic acid with photodynamic therapy (Motta and Monti, 2007). Corticosteroids and cytotoxic agents are often ineffective unless there is a coexistent, secondary vasculitic process. There have been reported cases of successful IVIG use in livedo vasculitis (Amital et al., 2000) and leukocytoclastic vasculitis, but the true efficacy of IVIG for aPL-related skin ulcers is unknown. Similarly, the efficacy for plasma exchange for this problem is not known, although its use has been described in patients with widespread cutaneous necrosis (Frances et al., 2005). Autologous skin transplantation can be used to treat large skin defects; in this case high-dose prednisone and immunosuppressive agents (e.g. cyclosporin A) are recommended for graft acceptance in the presence of concomitant biopsy-proven vasculitis (Fiehn et al., 2001).

Based on our limited experience with rituximab, it has been an effective agent of recalcitrant ulcers due to aPL-related thrombotic vasculopathy. In a case series describing five patients with primary APS who received rituximab for recalcitrant disease, two of the patients had such skin ulcers. In both of these patients, the ulcers healed after treatment with rituximab (Tenedios et al., 2005a). Skin ulcer is one of the endpoints that are evaluated in the RITAPS trial (see Section 5.1 for further discussion). The need for a systematic approach to defining the efficacy of these treatments is clear.

In summary, despite lack of controlled data, the current management of aPL-related skin ulcers includes anticoagulation with or without immunosuppressive treatment.

10. Lupus anticoagulant hypoprothrombinemia syndrome

Although lupus anticoagulant hypoprothrombinemia syndrome (LAC-HPS) is rare, with fewer than 50 case reports (Vinet et al., 2006), patients with positive LAC tests can present with life-threatening bleeding complications due to concomitant hypoprothrombinemia. The management is most often corticosteroids but corticosteroid-resistant cases have been reported. In LAC-HPS patients, surgical procedures can result in serious bleeding complications.

In LAC-HPS patients, hemorrhagic episodes result from non-neutralizing antibodies directed against prothrombin (factor II) (Erkan et al., 1999). In fact, although these antibodies are demonstrable in 67–74% of LAC-positive SLE patients (Edson et al., 1984; Fleck et al., 1988), severe life-threatening bleeding complications occur only when accelerated clearance of complexes cannot be compensated (Bajaj et al., 1983) and this entity is called LAC-HPS. The syndrome is more common in children and usually associated with SLE (Baca et al., 2002). In LAC-HPS, the antibodies against prothrombin usually have high

affinity, although low-affinity antibodies have been also reported (Bajaj et al., 1983). The trigger of the prothrombin antibody, the association of hypoprothrombinemia with LAC, and the reason(s) why patients develop clinical manifestations are unknown. Quinidine and phenytoin-induced hypoprothrombinemia have been reported in two different LAC-positive patients (Harrison et al., 1987; Clauser et al., 2007).

The diagnosis is based on the demonstration of the significant prolongation of the prothrombin time (PT) and low prothrombin activity in the setting of severe bleeding with positive LAC test. In patients with unknown LAC test status, the STACLOT® test may be more accurate than the DVV Confirm® test (confirmatory steps for LAC testing) as the STACLOT test includes normal platelet-poor human plasma that corrects prothrombin deficiency (Baca et al., 2002).

Corticosteroids are the first-line treatment in LAC-HPS and are believed to decrease the clearance of the prothrombin–antiprothrombin antibody complexes (Bajaj et al., 1983). Most cases respond successfully to corticosteroids but not to vitamin K, fresh frozen plasma, or blood transfusions (Erkan et al., 1999). Cases in which azathioprine or cyclophosphamide is required due to recurrence of coagulopathy during corticosteroid tapering have been reported (Lillquist et al., 1978; Eberhand et al., 1994; Taddio et al., 2007).

In LAC-HPS patients, a bleeding episode does not rule out future thrombosis (Vinet et al., 2006) and during pregnancy and surgical procedures, patients are at simultaneous risk for both bleeding and vascular thrombotic complications. Use of heparin or warfarin in LAC-HPS patients for vascular thrombosis can be problematic but, at times, necessary.

Life-threatening bleeding complications can occur in LAC-HPS patients during the perioperative period (Shaulian et al., 1981). Thus, PT should be followed perioperatively, as a significant elevation can be a warning sign of future bleeding. Usually, PT is significantly prolonged when prothrombin level is less than 30% of normal (Simel et al., 1987). The risk of bleeding in LAC-HPS patients, however, does not correlate with the degree of the prolongation of the PT as it may be artifactually prolonged in the presence of a positive LAC test. In patients with persistent hypoprothrombinemia, preoperative immunosuppression should be considered; rituximab with plasma exchange was successfully used in a patient to normalize the prothrombin level; interestingly corticosteroid–cyclophosphamide and corticosteroid–rituximab combinations had no effect on the prothrombin levels in this patient (Raflores et al., 2007).

During pregnancy of LAC-HPS patients, although a history of bleeding cannot be considered an APS-related vascular event, prophylactic treatment with aspirin can be started due to the presence of LAC and a successful fetal outcome has been reported (Erkan et al., 2001). Alijotas-Reig and Ferrer-Raventos reported two LAC-positive patients with steroid-responsive postpartum bleeding; in both patients, prothrombin activities were normal (Alijotas-Reig and Ferrer-Raventos, 2004). Of note, pregnancy results in a considerable increase in prothrombin levels (Lockshin et al., 1999), which should be part of the diagnostic and therapeutic considerations in pregnant LAC-HPS patients.

In summary, LAC-HPS is rare but can result in serious complications, especially if the diagnosis and treatment is delayed. Thus, in the setting of severe bleeding with positive LAC test and significant prolongation of the PT or activated partial thromboplastin time (aPTT), one should always consider the possibility of LAC-HPS and proceed with coagulation factor work-up.

11. Diffuse alveolar hemorrhage

There is a diverse clinical spectrum of pulmonary manifestations in APS, including pulmonary embolism and infarction, thromboembolic pulmonary hypertension, fibrosing alveolitis, pulmonary vasculopathy, and diffuse alveolar hemorrhage (DAH). Patients with DAH can present with dyspnea, pleuritic chest pain, fever, hemoptysis, hypoxemia, and, in the more severe cases, respiratory failure. Patients are often anemic and thrombocytopenic. The chest X-ray typically reveals

bilateral alveolar infiltrates that should resemble pneumonia.

Diffuse alveolar hemorrhage is characterized by bleeding into the acinar portion of the lung. Histopathologically, biopsy specimens reveal microvascular thrombosis, septal thickening due to edema, neutrophilic septal infiltrates, and extravasation of red blood cells into the alveolar spaces. The underlying pathophysiology is still not clear, but there is a disruption of the pulmonary or bronchial circulation with subsequent bleeding into the alveolar spaces. A proposed theory is aPL-induced upregulation of endothelial cell adhesion molecules with subsequent neutrophil recruitment and migration into the alveolar septae, the release of proteases, septal inflammation, alveolar disruption, tissue destruction, and hemorrhage (Deane and West, 2005). It is not clear whether the pathophysiology has a thrombotic component. There are adequately anticoagulated APS patients presenting with acute DAH, suggesting a vasculitic and non-thrombotic nature of DAH.

Treatment for DAH secondary to APS depends on the severity of the clinical presentation. Corticosteroids are the first-line treatment with mild cases requiring only oral steroids and more severe cases requiring pulse intravenous corticosteroids. Often, patients have recurrence of disease in the setting of a corticosteroid tapering or have episodes of bleeding while on steroids. In these situations, steroid-sparing agents that have been used include plasmapheresis, cyclophosphamide (oral or intravenous), IVIG, rituximab, azathioprine, cyclosporine, and/or mycophenolate mofetil (Deane and West, 2005; Gertner, 1999). Of note, rituximab (375 mg/m^2) every 2 weeks for 6 weeks has been successfully used to treat DAH in a 29-year-old SLE patient after she failed a combination of intravenous prednisolone 500 mg/day, cyclophosphamide, and plasmapheresis (Nellessen et al., 2008). Management of anticoagulation can be challenging in APS patients with history of clotting and active bleeding. As significant bleeding from the lung can be fatal, anticoagulation is generally held and reinstituted once bleeding resolves.

In summary, long-term management of patients with DAH secondary to APS will likely require immunosuppression. In addition, clinicians must delicately balance the risk of bleeding with the need for anticoagulation.

12. Dysfunctional uterine bleeding

Although, menorrhagia is among the minor bleeding complications seen in patients on anticoagulation, the management can be a challenge for physicians (Palareti et al., 1996). In a population without a hypercoagulable state, antifibrinolytic agents like tranexemic acid or aminocaproic acid, and estrogen-containing oral contraceptive pills are generally used. In patients with persistently positive aPL with or without APS, these medications are contraindicated due to associated risk of thrombosis.

Intramuscular or oral progestin agents can be considered in aPL-positive patients. Their use is associated with reversible osteoporosis, irregular bleeding, weight gain, acne, and prolonged return of fertility after discontinuation (Sammaritano, 2007). In the general population, oral and injectable progestin-only contraceptives have shown no increased risk of stroke, venous thromboembolic disease, or acute myocardial infarction (WHO, 1998). However, when progestagens are used for the treatment of menstrual disorders, there is an association with increased risk for venous thromboembolic disease (VTE). In a study by Vasilakis et al. the adjusted relative risk for venous thromboembolic disease in patients using oral progestagens for menstrual disorders was 5.3 with a confidence interval of 1.5–18.7. The relatively wide confidence interval reflects the small number of cases seen. This risk was not seen when the progestins were used for oral contraception which may reflect the different dosages used for the different indications (Vasilakis et al., 1999) or it may reflect an aspect of the different indications themselves (Poulter et al., 1999). Labeling of these medications reflects this finding, although in practice they are still sometimes used in patients with known thrombophilia.

A new consideration is the use of the levonorgestrel-releasing intrauterine device (LNG-IUS or

Mirena coil). The efficacy of this device in the treatment of idiopathic menorrhagia has been illustrated in previous trials. Lähteenmäki et al. randomized patients with excessive uterine bleeding (such that hysterectomy was planned) to continuation of present medical therapy or insertion of LNG-IUS as they awaited their surgeries. After six months, 64.3% of those patients who had received the LNG-IUS decided to cancel their surgery compared with 14.3% of patients on continued medical therapy ($p<0.001$) (Lähteenmäki et al., 1998). In a survey of 17 anticoagulated patients from an APS clinic who used the LNG-IUS, vaginal bleeding was reduced in 58.8% and amenorrhea occurred in 23.5%. Forty-seven per cent felt very satisfied with the device and 23.5% felt somewhat satisfied (Pisoni et al., 2006). In this small study pelvic infection was not seen, but increased risk of pelvic infection is a consideration, especially in patients who are on immunosuppressive medication.

In summary, although dysfunctional uterine bleeding remains a treatment challenge for patients with APS, new strategies exist to manage patients without surgery. The safety of these strategies in specific patient populations remains to be assessed.

13. Perioperative medical management of APS patients

Antiphospholipid syndrome patients are classified in the very high risk category for venous thromboembolism during the postoperative period (Geerts et al., 2001). Perioperative thromboses can occur due to: (a) withdrawal of warfarin (Asherson et al., 1985); (b) increased hypercoagulability despite ongoing, optimal warfarin or heparin therapy (Bick et al., 1999); and (c) catastrophic exacerbation of APS (Yamamoto et al., 2000). In addition to thromboses, life-threatening bleeding complications can occur during the perioperative period due to: (a) excessive anticoagulation; (b) thrombocytopenia (Asherson et al., 1999); and (c) associated coagulation factor deficiencies such as high-affinity anti-prothrombin (factor II) antibodies (Erkan et al., 1999).

When patients with persistent and significant aPL profiles (with or without APS diagnosis) undergo a surgical procedure, the most effective pharmacological methods should be combined with physical methods like intermittent venous compression, and patients should be closely observed for the signs and symptoms of thrombotic clinical events. Furthermore, perioperative strategies should be clearly identified before any surgical procedure, periods without anticoagulation kept to an absolute minimum, and any deviation from a normal course be considered a potential disease-related event.

Recommended standard antithrombotic regimens for high-risk patients should be the minimum administered dose in APS patients (Madan et al., 1997). No studies are available for the most accurate dosing; it is possible that the current recommended doses result in ''under-anticoagulation'' of APS patients. Timing of the anticoagulation is also crucial in APS patients in order to keep periods without anticoagulation to an absolute minimum. The practice patterns can vary based on the type of procedure and surgeons' experience, but the anticoagulation should be re-started as soon as possible postoperatively. The literature on the perioperative medical management of APS is limited and based on case reports and retrospective studies. Despite this, strategies that may guide physicians in their preoperative, intraoperative, and postoperative management of APS patients are summarized in Table 3 (Erkan et al., 2002b).

Some specific perioperative situations merit attention:

- Patients with APS undergoing cardiovascular procedures have increased morbidity and mortality, especially those where cardiopulmonary bypass is used. A review of the literature of APS patients undergoing cardiac surgery showed 7% early deaths and 12% late deaths after a mean follow-up period of less than 3 years. Morbidity was high as well, with only 42% of patients having an uneventful recovery. In valve surgeries about 20% of patients had valve-related complications (Gorki et al., 2008). The authors suggested application of less protamine than usual or none at all,

Table 3
Recommendations for the perioperative medical management of the antiphospholipid syndrome (APS) patients

Preoperative assessment	• Prolonged activated partial thromboplastin time (aPTT) and/or slightly prolonged prothrombin time (PT) when known to be due to APS are not contraindications for surgical procedures • Platelet count greater than 100×10^9 L due to APS requires no specific therapy; thrombocytopenia does not protect against thrombosis • Surgical and interventional procedures should be the last option in the management of APS patients
Perioperative considerations	• Minimize intravascular manipulation for access and monitoring • Set pneumatic blood pressure cuffs to inflate infrequently to minimize stasis in the distal vascular bed • Avoid tourniquets • Maintain high suspicion that any deviation from a normal course may reflect arterial or venous thrombosis
Perioperative anticoagulation	• Keep periods without anticoagulation to an absolute minimum • Employ pharmacological and physical antithrombosis interventions vigorously and start immediately before the operation, continuing until the patient is fully ambulating • Be aware that antiphospholipid syndrome patients can develop recurrent thrombosis despite appropriate prophylaxis • Be aware that current conventional doses of anti-thrombotic regimens can result in "under-anticoagulation"; APS patients may benefit from an aggressive approach with higher doses than standard doses • Manage APS patients whose only clinical manifestation is pregnancy morbidity as if they had vascular thrombosis
Renal transplant patients	• Perioperatively, anticoagulate all APS patients (history of thrombosis) undergoing renal transplant aggressively • Consider pretransplant plasma exchange in high-risk APS patients undergoing living donor transplantation, even if there are no data demonstrating that this approach changes the long-term outcome • Strongly consider perioperative anticoagulation in asymptomatic (no history of thrombosis) aPL-positive patients

Note: aPTT, activated partial thromboplastin time; PT, prothrombin time; APS, antiphospholipid syndrome; aPL, antiphospholipid antibodies.

Source: Adopted from Erkan et al. (2002b).

potentially increasing the target PTT, and paying scrupulous attention to detail pre- and postoperatively.

- Patients with APS undergoing renal transplantation are at high risk for renal allograft failure; despite anticoagulation, graft failure may occur in transplant recipients (Vaidya et al., 2004). Based on case reports, there may be a role for concomitant plasma exchange in these high-risk patients (Ruffatti et al., 2007). Even without the history of thrombosis, aPL in lupus patients undergoing renal transplantation increases graft failure, morbidity, and mortality (Stone et al., 1999; McIntyre and Wagenknecht, 2001; Raklyar et al., 2005). In a non-SLE population, the role of aPL in graft failure is controversial, with some studies showing a correlation between asymptomatic aPL positivity and graft failure (Wagenknecht et al., 1999) and others not finding this relationship (Forman et al., 2004). It has been reported that anticoagulation begun before or at the time of kidney transplantation reduces posttransplant thrombosis (Vaidya et al., 2000). In the absence of definitive data, we recommend prophylactic perioperative heparin for aPL-positive patients undergoing renal transplant, although the risks and benefits must be weighed in a given clinical situation.

14. Endovascular procedures: cardiac stents and inferior vena cava filters

Patients with APS have an especially high risk of thrombosis with any procedure or instrumentation. The clinical dilemma often arises when patients need a life-saving intervention such as a coronary stent or an inferior vena cava (IVC) filter, yet are at increased risk of forming a clot around the foreign body.

Repeated acute stent thrombosis has been described in an APS patient (Weissman and Coplan, 2006). A 46-year-old woman with APS had four paclitaxel drug-eluting stents placed and

presented two months later with stent restenosis, despite 100% compliance with aspirin and clopidogrel. She was restented and 2 days later presented with a myocardial infarction and occlusion of multiple stents. This patient eventually had coronary artery bypass graft (CABG) surgery. Postoperatively, the patient was anticoagulated with warfarin with a goal INR of 2.5. The role of post-stent and post-CABG anticoagulation is not clear, although it may be of potential benefit and should be considered in APS patients.

Although anticoagulation is the first-line treatment for thromboembolic disease, it may be contraindicated when bleeding occurs or patients may develop venous thromboemboli despite anticoagulation. In these situations, IVC filters are often considered to prevent the embolization of a thrombus from the deep venous system to the pulmonary arteries. The utility of IVC filters in APS patients is controversial as both deep vein thrombosis (DVT) and IVC thrombosis may result from the procedure itself (Cherian and Gertner, 2005). Two published case reports in APS patients revealed recurrent pulmonary emboli despite IVC filter placement with clot both proximal and distal to the filter (Ebato et al., 2002; Cherian and Gertner, 2005). Ebato and colleagues again demonstrated the failure of an IVC filter in an APS patient. They discussed a 62-year-old woman with APS and an IVC filter implanted 5 years earlier who presented with tricuspid valve thrombus and pulmonary emboli (Ebato et al., 2002). Also, IVC filters may fail in APS patients and may not protect from pulmonary emboli if collateral vessels develop around the filter or if a thrombus is present on the proximal side of the filter.

In summary, the role of IVC filters in APS patients remains controversial as they may not be helpful and may actually aid in the formation of thrombus.

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CHAPTER 19

Future of the Antiphospholipid Syndrome

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1. Introduction

Since the first report of lupus anticoagulants over 50 years ago, much progress has been made in the characterization of antiphospholipid autoantibodies (aPL) and the clinical manifestations associated with them. Progress has been particularly rapid in the last decade, as evidenced by the need for a third revision of this text in 8 years. The many recent advances in our understanding of the antiphospholipid syndrome (APS) have been elegantly discussed in the preceding chapters. In this chapter, we will discuss issues that remain unresolved in APS and speculate on how ongoing research could impact our understanding and management of this syndrome in the near future.

2. Magnitude of the problem/Clinical implications

Before discussing key areas for future research, one should consider the future of APS clinically and its impact on patients and physicians. The prevalence of APS in the general population has not been well established. It can, however, be roughly estimated from the incidence of venous and arterial thrombosis, and pregnancy loss. The overall incidence of venous thromboembolism in the United States is rising and is estimated to be around 900 000 per year (Heit et al., 2005). Given limitations in the process by which this information is gathered, there is wide consensus that the true number is likely to be significantly higher than the quoted estimate. It is estimated that antiphospholipid antibodies are associated with 10–15% of venous thromboembolism (VTE) (Ginsburg et al., 1992; Schulman et al., 1998) and 10–18% (Nencini et al., 1992; Levine et al., 2004) of patients with stroke. The incidence estimates among women with pregnancy loss vary more widely. Of the 500 000 cases of recurrent pregnancy loss in the United States annually, over 20% are thought to be related to aPL, the most common identifiable cause for this condition (Bick, 2008). However all of these numbers should be interpreted with caution given the limitations of the studies. Based on the above mentioned incidence rates, one can estimate that APS will be the major underlying cause for about 90 000–135 000 cases of venous thromboembolism, 60 000–100 000 cases of stroke, and over 100 000 cases of recurrent pregnancy loss annually in the United States. This is the grim future of APS for patients and their families. These estimates, combined with the significant morbidity and mortality associated with APS, underscore the urgent need for advances in laboratory testing, risk assessment, and management.

3. Pathophysiology of the antiphospholipid syndrome

While significant progress has been made, many questions remain regarding our understanding of

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the pathological mechanisms underlying thrombosis and pregnancy loss in APS. Antiphospholipid antibodies are probably best considered as risk factors for thrombosis, rather than the proximate cause for an acute thrombotic event. Clinical observations and animal models suggest that the presence of these antibodies alone is insufficient to cause adverse outcomes and a second, precipitating event is often necessary to trigger thrombus formation. In other words, aPL contribute to a thrombotic diathesis, and the specific time and location of a thrombotic event is likely determined by one or more precipitating events or "second hit" (Vega-Ostertag et al., 2004; Fischetti et al., 2005).

The contribution of inflammation and endothelial activation to the pathogenesis of APS is increasingly apparent (Raschi et al., 2003; Bohgaki et al., 2004; Ferrara et al., 2004; Zhou et al., 2004; Vega-Ostertag et al., 2005). Recently, activation of the complement cascade has been shown to play a key role in animal models of aPL-mediated thrombosis (Fischetti et al., 2005; Ritis et al., 2006) and pregnancy loss (Girardi et al., 2004; Redecha et al., 2007). These recent advances are likely to impact management of patients with APS as discussed below.

4. Clinical spectrum of the antiphospholipid syndrome

The spectrum of clinical manifestations attributed to APS is broadening as discussed in the preceding chapters. In the original APS consensus classification criteria proposed in 1999 (Wilson et al., 1999), the clinical manifestations of APS were limited to arterial and venous thrombosis, and recurrent pregnancy loss. It is important to recognize that these classification criteria were developed to standardize inclusion criteria for subjects in clinical studies, and were not intended as diagnostic criteria for APS in individual patients or to reflect the total clinical spectrum of this syndrome. Clinical features such as thrombocytopenia and livedo reticularis were excluded from the classification criteria. It is, however, well recognized that APS is a multisystem disorder that can manifest a myriad of clinical features, some of which may not be directly related to thrombosis. For instance, neuropsychiatric manifestations including cognitive defects are associated with aPL and do not appear to be thrombotic (Tektonidou et al., 2006). Similarly, aPL are also associated with cardiac manifestations such as myocardial dysfunction (cardiomyopathy, diastolic dysfunction) and valvular disease (valve thickening/fibrosis, Libman–Sachs endocarditis). The association of thrombotic microangiopathies with APS has also been well described (Espinosa et al., 2004). Indeed, new subsets of APS such as microagiopathic APS (MAPS) and probable APS (PRE-APS) have recently been proposed (Asherson, 2006). Better understanding of the spectrum of clinical manifestations associated with aPL could lead to the development of useful classification criteria for APS that take into account the multiple organ systems affected by this disease.

5. Clinical laboratory testing for antiphospholipid antibodies

The diagnosis or classification of APS is based on the detection of one or more aPL: lupus anticoagulants detected in various coagulation assays, and anticardiolipin and/or anti-β_2 glycoprotein I (β_2GPI) antibodies typically detected by enzyme-linked immunosorbent assays (ELISAs) (Miyakis et al., 2006). A preponderance of evidence suggests that the major, clinically relevant autoantigens in APS are β_2GPI and prothrombin. With additional clinical experience and standardization efforts, it is likely that the use of anti-β_2GPI ELISAs and new anti-prothrombin ELISAs will increase. In contrast, use of anticardiolipin ELISAs may decrease. The latter seem to have a greater incidence of low positive results that may not be associated with an increased risk of thrombosis or other clinical manifestations of APS. A recent report showing improvement in the performance of commercially available anticardiolipin and anti-β_2GPI assays with good concordance between different kits (Andreoli et al., 2007) is encouraging. In contrast, ELISAs to detect antibodies to a range of other phospholipids continue to be difficult to interpret due to lack of standardization. With the possible exception of

antibodies to phosphatidylethanolamine, these tests do not appear to offer useful clinical information beyond that obtained from anticardiolipin/anti-β_2GPI ELISAs and lupus anticoagulant assays.

Interpretation of aPL assays remains clinically challenging. Rather than viewing these as diagnostic tests for APS, it may be preferable to consider the presence of aPL as a risk factor that increases the odds of thrombosis, pregnancy loss, and other manifestations of APS. In other words, the risk of thrombosis is increased in individuals with persistent moderate to high levels of anticardiolipin/anti-β_2GPI antibodies or a positive lupus anticoagulant. Some individuals with these antibodies will experience thrombosis and some will not. This is analogous to the increased risk of thrombosis in individuals with inherited thrombophilia. For example, individuals who are heterozygous for factor V Leiden have a significant increased risk of thrombosis, although the majority will never experience a thrombotic event. Thus, the distinction between patients diagnosed with APS vs. individuals with aPL who have not yet had a clinical manifestation of APS may be less important than the current classification criteria imply. A wide range of factors, some directly related to aPL (specificity, isotype, titer) and many independent of aPL, probably play a role in determining whether or not someone with antibodies will develop clinical manifestations of APS. In future studies, emphasis should be placed on a more complete and accurate assessment of the risk associated with specific aPL profiles, antibody titer, and antibody persistence.

6. Areas of uncertainty regarding patient management

Numerous questions remain regarding the optimal treatment of patients with aPL and APS. Important areas that should be addressed in future studies are as follows:

6.1. Fluctuating antibody titers

It is well recognized that the aPL titers fluctuate in a subset of VTE patients and in some instances become negative. Do these patients truly have APS? Is the risk for adverse events in this subset comparable to that of patients with persistently positive antibodies? How should we treat patients with antibodies that are only intermittently detected on serial testing?

6.2. Duration of anticoagulation

The duration of anticoagulation for patients with thrombosis that meet criteria for APS is debatable. Current recommendations suggest indefinite anticoagulation (Buller et al., 2004). However, in a subset of APS patients, aPL titers significantly decrease over time or become undetectable during long-term follow-up. The optimal duration of anticoagulation in such patients is unknown. Could anticoagulation be safely discontinued in these patients? In addition, could markers of risk for recurrence, such as elevated D-dimer levels or presence of residual vein thrombosis, be utilized in determining duration of anticoagulation? These markers have been demonstrated to be useful in estimating risk for recurrence of VTE in the general population (Poli et al., 2008). These issues need to be explored using well-designed prospective clinical trials.

6.3. Intensity of anticoagulation

There are also unresolved questions regarding the optimal treatment of patients with APS and arterial thromboses. While there is good evidence from clinical trials to support moderate intensity anticoagulation (INR between 2 and 3) with vitamin K antagonists in patients with VTE, controversy continues regarding the optimal management of patients with arterial thrombosis, particularly in those with non-cardioembolic events. Is moderate intensity adequate for these patients or do they require high-intensity anticoagulation (INR between 2.5 and 3.5)? Current consensus recommendations in this setting are not based on strong evidence (grade 2 C).

Table 1
Oral anticoagulants currently in clinical trials VTE, venous thromboembolism

Drug name	Mechanism of action	Prophylaxis of VTE	Treatment of VTE
A: Drugs in phase III trials			
Dabigatran	Oral direct thrombin inhibitor	a. RENOVATE trial, Phase III study in post arthroplasty prophylaxis, completed (Eriksson et al., 2007b) b. REMEDY trial, phase III, extended prophylaxis post treatment, ongoing	RECOVER trial, Phase III trial ongoing
Rivaroxaban	Oral Xa inhibitor	RECORD trials, phase III studies in post-arthroplasty prophylaxis, completed (Eriksson et al., 2007a), (NCT 00329628; NCT 00332020).	EINSTEIN trials, phase III, ongoing (NCT 00440193; NCT 00439777; NCT 00439725)
Apixaban	Oral Xa inhibitor	a. ADVANCE-1 trial, ongoing, phase III (NCT 00371683) b. ADOPT trial, ongoing, phase III (NCT00457002)	Botticelli-DVT trial, phase II, completed (Buller, 2007)
B: Other oral Xa inhibitors in the pipeline a. LY517717 b. Betrixaban c. DU-176b d. YM150			

Source: Adapted From Turpie (2008).

6.4. Anticoagulant versus antiplatelet therapy

In the subset of patients with arterial thrombosis discussed above, the role of antiplatelet agents versus anticoagulation for secondary prophylaxis needs to be evaluated using clinical trials.

7. Newer anticoagulants

The development of alternatives to vitamin K antagonists has been the focus of pharmaceutical research for some time. These efforts are finally bearing fruit based on the results of recent clinical trials. New anticoagulants directed against coagulation factors II and X offer the distinct advantage over vitamin K antagonists in that they may have few drug interactions, are not affected by dietary factors, and do not require monitoring. Details of these agents including their targets and stage of development are listed in Table 1. The results of the RECORD trials evaluating rivaroxaban, an oral anti-Xa agent, versus enoxaparin in thromboprophylaxis following orthopedic surgery, were reported recently (Eriksson et al., 2007a). These showed rivaroxaban to be equivalent if not superior to enoxaparin in this setting with a 70% decrease in the relative risk for VTE. This drug is currently undergoing phase III clinical trials in the treatment setting. It is likely that such agents will be available for clinical use within the next few years and have the promise of greatly simplifying anticoagulation therapy for all patients with thromboses.

8. Novel therapeutic approaches

Novel treatment approaches that could potentially be useful in the treatment of APS in parallel with anticoagulation or antiplatelet therapy deserve mention. The role of targeted immune modulation using monoclonal antibodies has revolutionized the management of many autoimmune diseases. While they have not been formally evaluated for the treatment of APS, there are anecdotal reports of their use in this setting. In two small case series administration of the anti-CD20 monoclonal antibody rituximab was associated with clinical improvement, the absence of recurrent thrombotic events, and lower aPL titers (Rubenstein et al.,

2006; Trappe et al., 2006). This agent should be evaluated prospectively in randomized clinical trials. Another novel approach is the depletion of pathogenic anti-β_2GPI autoantibodies using a specific B-cell toleragen, LJP1082. In a phase I/II clinical trial this agent appeared to be well-tolerated (Cockerill et al., 2004). The increasing evidence for a role for complement activation in the pathogenesis of APS raises the possibility of the use of selective complement inhibition as a therapeutic strategy. Two such agents are currently available, both of which target complement C5, thereby preventing the generation of C5a and the C5b-9 membrane attack complex. Pexelizumab, a recombinant humanized single-antibody chain fragment, has undergone phase III clinical trials in percutaneous coronary interventions and coronary artery bypass grafting (Armstrong et al., 2007; Eikelboom and O'Donnell, 2007). In both situations, the drug conferred no benefit, although one of the two studies was significantly underpowered. The second drug, eculizumab, is a recombinant, fully humanized monoclonal antibody that has been studied extensively in paroxysmal nocturnal hemoglobinuria, where it has been shown to offer significant benefit (Hillmen et al., 2007; Brodsky et al., 2008). There is as yet no data on the use of either of these agents in patients with APS but this is an interesting prospect that deserves further evaluation. In addition, it has been shown recently that heparin may have complement inhibitory properties in addition to its anticoagulant effects (Girardi et al., 2004). It has been proposed that the beneficial effects of heparin in patients with history of recurrent pregnancy losses may be related to the complement inhibitory rather than the anticoagulant properties per se. However, it is not known whether the low-molecular-weight heparins also have such properties. This is important as low-molecular-weight heparins are increasingly being used for prophylaxis in pregnant patients with APS.

9. Better patient education and advocacy

Finally, an important and often overlooked aspect in the care of patients with this chronic disease is patient support and advocacy. Venous thromboembolism awareness and prevention has been a focus of the National Institutes of Health and the Center for Disease Control and Prevention in the year 2007. A new multidisciplinary group, the Venous Disease Coalition, was inaugurated recently. This is a collaborative network of professional and public organizations whose shared goal is to increase public and physician awareness about venous diseases including VTE. Of particular relevance to patients with APS are the Hughes Syndrome Foundation (www.hughes-syndrome.org) and the APS Foundation of America (www.apsfa.org), patient advocacy groups that are active in the support of patients with APS. A similar organization that is very active in patient education and advocacy pertaining to all thrombophilias is the National Alliance for Thrombosis and Thrombophilia (www.nattinfo.org).

10. Summary

In the coming years, it is expected that the number of patients with APS, and the number recognized as having APS, will grow. Despite significant advances in our basic knowledge about APS, many important scientific questions and clinical issues remain. Hopes for the future include the following: (1) a better understanding of the molecular pathophysiology of APS will lead to the identification of new and potentially safer targets for therapy, (2) new oral anticoagulants will offer significant benefits over warfarin with respect to both safety and the need for monitoring, (3) a better understanding of the risks associated with aPL will allow clinicians to identify high-risk patients and use therapeutics agents more effectively, and (4) better education and advocacy will lead to greater awareness of APS among primary care physicians and will assist APS patients and their families in facing this difficult disease.

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