Doruk Erkan · Michael D. Lockshin
Editors

Antiphospholipid Syndrome

Current Research Highlights and Clinical Insights

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To:

ARIN, AYDIN, & RACHEL

(DE)

AMANDA & JANE

(MDL)

Preface

"Advancing the Field Together" – The Journey that started in planning for the 15th International Congress on Antiphospholipid Antibodies continues with a State-of-the-Art Antiphospholipid Syndrome book

The International Congress on Antiphospholipid Antibodies (aPL) takes place every three years; its purpose is to discuss aPL and antiphospholipid syndrome (APS) research and clinical care. The Congress is a venue in which scientists and clinicians, from multiple disciplines, present and review the most recent innovative and important research to improve understanding of the syndrome, prioritize research questions, and set a roadmap for future research.

The 15th International Congress on aPL took place on September 21–24, 2016, in North Cyprus, having been relocated from the original congress location of Istanbul, Turkey[1]. The Congress offered a comprehensive program, including evidence-based state-of-the art presentations from 70 internationally recognized physicians and scientists. At the Congress, there were 24 main sessions including 4 from different task forces, 147 oral and poster abstract presentations, 14 meet-the-expert sessions, 2 satellite symposia, and 1 patient education workshop; the objectives of which were to discuss the biology and new mechanisms of the disease, describe ongoing and planned clinical trials, explore potential new treatments, and strengthen established and/or create new international collaborations for both basic and clinical research in the field.

A novel aspect of the Congress was that multiple teams, chaired by Scientific Planning Committee members, used evidence-based literature reviews and expert discussions to answer specific predefined aPL/APS questions. These teams included points of view from experts in rheumatology, hematology, cardiovascular medicine, obstetrics, neurology, and immunology. The Scientific Planning Committee members also chaired the congress sessions and supervised completion of the reports that are the bases of the chapters of this book.

The journey that had started as a planning exercise for the 15th International Congress on aPL did not stop in September 2016, when the Congress ended, but

[1] www.apsistanbul2016.org.

continues with this state-of-the-art book. This book is presented in six sections, each section focusing on a different aspect of aPL/APS: history; basic science; clinical and diagnostic features; current and future therapies; task forces; and patient education. We want to thank all the authors who participated in this journey, and hope that this book will add value to the field of thrombosis and APS.

New York, NY, USA

Doruk Erkan*
Michael D. Lockshin**

* Congress Chair

** Congress Honorary Chair

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Doruk Erkan
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I want to express my personal gratitude to Dr Michael D. Lockshin for his invaluable guidance and support over the years.

Doruk Erkan

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Part I
Historical Aspects of Antiphospholipid Syndrome

Chapter 1
History of Antiphospholipid Antibody

Michael D. Lockshin and E. Nigel Harris

Introduction

If one adds molecular and biological science to clinical description, the history of antiphospholipid antibody (aPL) and antiphospholipid syndrome (APS) contains nine phases (Table 1.1). We base this history on personal memory (primarily that of MDL) and on conversations with the early North American leaders, Samuel Rapaport, Lawrence Shulman, Sandor Shapiro, Donato Alarcon-Segovia, and Carl Alving; others have seen the history differently [1–3]. The nine phases emphasize mechanistic studies as well as clinical ones. Although the North American view necessarily has a New World bias, we do not wish to understate the important contributions from Europe, Asia, South Africa, Australia, and Central and South America—it's just that we have less personal experience with them. This history omits many investigators, papers, and contributions. For those omissions I (MDL) apologize.

1906–1962: Identifying an Antigen, Associating the Antibody with Systemic Lupus Erythematosus

In 1905 August Paul von Wassermann, recognizing the spirochete, *Treponema pallidum*, to be the cause of syphilis, used complement fixation, an immunological test available in his era, to identify an antibody that binds the surface of the spirochete. Thus, he created a diagnostic test for syphilis that has lasted to this day [4]. It soon became apparent that Wassermann's antibody is also found in patients who do not

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Table 1.1 Major events in the history of antiphospholipid syndrome (APS)

	Years	Advances
1	1906–1962	Identifying an antigen, associating the antibody with systemic lupus erythematosus
2	1952–1980	Defining lupus anticoagulant, understanding a mechanism
3	1969–1985	Defining and measuring anticardiolipin
4	1963–1985	Defining a syndrome: thrombosis and pregnancy
5	1985–1989	Primary APS
6	1987–1991	A sub-syndrome: catastrophic APS
7	1990–1994	Animal models
8	1990–1999	β_2-Glycoprotein I
9	1996–2016	Criteria and international collaboration

have syphilis, a finding that led to the concept of biological false-positive test for syphilis (BFP). By the 1940s physicians recognized that the BFP is a signal of autoimmunity, since many patients with BFP also have systemic lupus erythematosus (SLE) or a related autoimmune disease [5]. Over the next two decades a team of physicians at the Johns Hopkins Hospital in Baltimore, Maryland (A. McGee Harvey, Lawrence E. Shulman, J.E. Moore, Philip Tumulty, and C.E. Conley) published a series of papers that described the relationship between BFP and SLE [6–9].

In 1941 Mary C. Pangborn, from the Division of Laboratories and Research, New York State Department of Health in Albany, New York, purified the syphilis antigen responsible for a positive Wassermann test. Because the antigen is a phospholipid extracted from beef hearts, she named it cardiolipin [10]. Forty years later this phospholipid reappeared at the center of the description of APS.

1952–1980: Defining Lupus Anticoagulant, Understanding a Mechanism

Independent discoveries by hematologists in the 1950s called attention to an association between BFP and a blood-borne inhibitor of coagulation, now termed lupus anticoagulant (LA). Lupus anticoagulant was initially thought to be a cause of hemorrhage [11, 12]. However, in 1959, a Mexican group led by L. Sánchez-Medal suggested that LA might be pro-thrombotic [13]. In 1963 E.J. "Walt" Bowie from the United States [14], and in 1967 Donato Alarcon-Segovia from Mexico [15], published clinical and mechanistic papers that more firmly established LA as a prothrombotic factor. In the 1970s an American group led by Samuel I. Rapaport demonstrated that the LA is an immunoglobulin of IgG or IgM isotype and its activity requires phospholipid [16, 17]. In 1980 Sandor Shapiro [18] (a mentor of Vittorio Pengo [19, 20]), in Philadelphia, demonstrated in vitro and presumably in vivo that

a molecule responsible for LA activity, which he isolated from a patient with Waldenstrom's macroglobulinemia, is an IgM antibody that, in an Ouchterlony precipitation assay, directly binds phospholipids.

Carl Alving, at the Walter Reed Institute in Washington, D.C., demonstrated in 1969 that lipids are immunogenic [21]. This finding, published in the biochemistry literature and unassociated with clinical concepts, lays fallow for a decade until, in 1980, Moshe Smolarsky, crediting Alving, described a radioimmunoassay that can identify antibodies to lipids [22].

1969–1985: Defining and Measuring Anticardiolipin

The association of BFP with SLE created a clinical conundrum because it is not a practical test. Uncommon in a community and more often true than falsely positive, to screen for syphilis is a poor way to screen for autoimmune disease. The test for LA is impractical, since it is also uncommonly present; as initially performed, it requires fresh plasma and an available, capable laboratory. Furthermore, the test for LA is not easily standardized. Thus a simpler way of identifying this phenomenon was required.

In 1983, E. Nigel Harris [23], working with Graham R. V. Hughes, used a radioimmunoassay, followed very shortly by a similar, more convenient, Enzyme-linked Immunosorbent Assay (ELISA) [24], to identify antibodies to cardiolipin. Use of this test markedly simplified the identification of aPL in patients with SLE. Through a series of conferences organized by Hughes, Harris, and Azzudin Gharavi, this test was standardized and internationalized, and the term antiphospholipid antibody (or anticardiolipin antibody) was first used in a clinical context [25]. The Hughes group generously shared their assay with others, allowing its widespread use and confirmation throughout the world. The first international meeting on aPL and its associated syndrome, APS (see below), organized by Hughes, took place in 1984 in London.

1963–1985: Defining a Syndrome (Thrombosis and Pregnancy)

The links between LA, SLE, and thrombosis have been known since the early 1960s. The association of LA with pregnancy loss was first published in 1975 by Inga Marie Nilsson [26], in English, in a not widely read Scandinavian journal. The same association was noted and published in French by J. P. Soulier and Marie-Claire Boffa in 1980 [27]. These papers were not widely cited until the radioimmunoassay and ELISA tests for anticardiolipin became available in 1983. Initially, aCL was thought to be a more rapid, more reliable, test for LA, that is, equivalent to LA,

and to impart the same clinical features. Clinical studies on the frequent occurrence of aCL in SLE patients rapidly led to the recognition that both thrombosis and pregnancy loss occur primarily in those who carry aCL/LA. This special form of SLE was soon called anticardiolipin syndrome, or lupus anticoagulant syndrome, or APS (the preferred term) [28].

The rapid use of the aCL ELISA worldwide led to an explosion of clinical papers that further defined the classical (thrombosis and pregnancy loss) manifestations of APS as well as nonclassical manifestations, like livedo reticularis, heart valve abnormality, thrombocytopenia, and cognitive dysfunction. Parameters of APS-associated pregnancy complication—early preeclampsia, growth restriction, thrombocytopenia, and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)—were described in more detail [29, 30].

1985–1989: Primary Antiphospholipid Syndrome

Within a few years many investigators, beginning with Ronald A. Asherson from Hughes' group in England in 1985 [31], described patients with APS who did not have diagnosable SLE. In 1987 Harris, in an editorial in which he described the essential features of APS, suggested that APS is an independent entity distinct from SLE. He called it the "syndrome of the black swan" [32]. 1989 brought several more reports from the Hughes group in England (Asherson [33] and Charles G. Mackworth-Young [34]) and Mexico (Alarcón-Segovia [35]) that dissociated APS from SLE and distinguished "primary" antiphospholipid syndrome (PAPS), in which SLE is not present, from "secondary" antiphospholipid syndrome (sAPS), in which APS accompanies SLE or a related illness.

1987–1991: A Sub-syndrome: Catastrophic Antiphospholipid Syndrome

In 1987 Asherson described a devastating complication of APS, multiple nearly simultaneous thromboses, now called catastrophic APS or CAPS [36]. Although some refer to CAPS as Asherson's syndrome, A. M. Harvey may have described CAPS in 1954 [9]. Two other papers published in 1987 also described CAPS [37, 38], as did a third paper in 1988 [39]. Asherson also described a signal manifestation of CAPS, adrenal infarction, in 1989 [40], the same year in which Alarcon-Segovia discussed the non-inflammatory vasculopathic pathology of CAPS [41]. R. D. Collins may have been the first to use the term "catastrophic," also in 1989 [42], and Stewart Greisman presented several cases with accompanying histologic pathology in 1991 [43]

1990–1994: Animal Models

Beginning in the 1990s, animal models enhanced our understanding of the mechanisms of APS. D. Ware Branch published a pregnancy loss model in 1990 [44], and Miri Blank and Yehuda Shoenfeld offered a similar model in 1991 [45]. Silvia Pierangeli described an animal model for thrombosis in 1994 [46]. The pregnancy and the thrombosis models have been widely and successfully exploited for mechanistic and treatment studies since that time. They remain in use today [47–49].

1990–1999: β_2-Glycoprotein I

In vitro the ELISA for aCL behaves aberrantly, becoming nonlinear when the tested serum sample is diluted with albumin or fetal calf serum instead of adult human or animal serum. This anomaly led investigators in Maastricht, Sydney, Sapporo, and Paris nearly simultaneously to identify a cofactor necessary for immunoglobulin binding to phospholipid [50–54]. This cofactor is now known to be β_2-glycoprotein I (apolipoprotein H, β_2GPI); aPLs are now thought to be primarily antibodies to β_2GPI and only secondarily, through β_2GPI, are they directed against phospholipids. Extensive molecular biological studies have defined the molecular and tertiary structures of β_2GPI, its five domains, its binding sites, and its antigenic sites.

Many papers on the biology of aPL and APS [55–57] focus on the molecular biology of β_2GPI, its antibodies and its receptors, its role in complement activation, endothelial and platelet activation, cytokines, pro- and antithrombotic proteins, placental biology, genomics and microbiomics, and other areas. The induction of aPL or antibody to β_2GPI by infectious agents, from viral (Epstein-Barr virus) to mycobacterial (leprosy), is a topic under active exploration. Alternatively, aPL may cross-react with antibodies to infectious agents. Treatments, including inhibitors of peptides on β_2GPI, complement, receptors, and B cells, competing antibodies, antiplatelet agents, and new and old anticoagulants, all target novel pathogenic pathways and mechanisms.

1996–2016: Criteria and International Collaboration

Largely through the efforts of the Hughes group in London, the field of APS has been collaborative since the beginning. International meetings have taken place every 2 or 3 years since 1984, the conference in Istanbul (relocated to North Cyprus) (www.apsistanbul2016.org) in 2016 being the Fifteenth. (Pierangeli and Harris summarized the history of these meetings through the Thirteenth [58].) The European Forum began in 1996 [59]; APS Alliance for Clinical Trials and International Networking (APS-ACTION), a group dedicated to clinical trials,

began in 2010 [60]. International consensus committees have devised clinical and laboratory criteria for classification, first in Sapporo in 1998 [61], with revision in Sydney in 2004 [62], and further efforts to develop new classification criteria under the auspices of the Fifteenth Congress [63]. Major hospital clinics focused on APS now exist in most areas of the world.

The Future

This history necessarily focuses on past events. Many important clinical questions—clear predictive ability, fully satisfactory treatment, possibly cures—are not yet available. The following questions require answers:

Why are APS and SLE linked?
Are obstetric and thrombotic APS the same or different diseases?
By what mechanisms do the chronic manifestations of APS (nephropathy, livedo, valvulopathy, cognitive dysfunction) occur?
What is the relationship between infection-induced and autoimmune-induced aPL?
What explains the familial nature of APS?
What explains APS' sex and racial distribution?
Are the best therapeutics target effector molecules, endothelial cells, platelets, coagulation factors, immunological molecules, or a combination of any of the above?
What distinguishes one antibody profile (single positivity) from another (double or triple)?
Why does aPL persist despite treatment?
What occurs that turns asymptomatic aPL into APS?

When these questions are answered, we will begin to believe that we will see an end to the devastation caused by APS.

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Part II
Basic Science Aspects of Antiphospholipid Syndrome

Chapter 2
Natural Proteins Involved in Antiphospholipid Syndrome

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Introduction

The antiphospholipid syndrome (APS) is characterized by antiphospholipid antibodies (aPL) in the plasma or serum of patients with thrombosis or pregnancy complications [1]. The APS is a misnomer, because the so-called aPL are directed not against phospholipids but against plasma proteins with affinity for anionic phospholipids. Autoantibodies against many different plasma proteins have been described. In this chapter we will enumerate these proteins, discuss the arguments why they are linked to the syndrome and discuss why these proteins become prothrombotic in the presence of autoantibodies.

Plasma Proteins Involved in Antiphospholipid Syndrome

β_2-Glycoprotein I

β_2-glycoprotein I (β_2GPI) is a 50 kDa plasma protein with increasing evidence that it has important roles in innate immunity and coagulation [2–4]. Many studies show that anti-β_2GPI antibodies (aβ_2GPI), either mouse monoclonal or patient derived, induce a prothrombotic phenotype in mice that have been primed either with lipopolysaccharide (LPS) or an injury to the vessel wall [5, 6]. Studies that separate aβ_2GPI-associated aPL from those in which aβ_2GPI are removed by affinity chromatography show that the prothrombotic effect of aPL is present only in the aβ_2GPI containing fraction [7].

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Further studies, characterizing correlations of the individual domains of β_2GPI with thrombosis and fetal loss, show correlation for both manifestations only with anti-domain I antibodies [8, 9]. Studies using aβ_2GPI depleted of anti-domain I antibodies further demonstrate that domain I antibodies are pathogenic, while antibodies against the other domains are not [10]. The major epitope for the autoantibodies is located within the region of amino acids arginine 39 and arginine 43 and a minor epitope involving lysine19 [11]. Exogenous human domain I can inhibit the prothrombotic phenotype in a mouse model of APS [12]. When arginine 39 is replaced by serine, the inhibitory potential of domain I is lost. These experiments in mouse models show conclusively that β_2GPI, in particular its first domain, is central to the pathogenesis of APS.

Prothrombin

Antiprothrombin antibodies are commonly found in patients with APS. Prothrombin is one of the major coagulation factors in blood. However, antiprothrombin antibodies do not correlate with thrombosis. A recently developed assay that measures autoantibodies against prothrombin complexed with phosphatidylserine (PS) shows better correlation of these antibodies with thrombosis [13]. Two studies show that antiprothrombin antibodies are prothrombotic in mouse models of APS [14, 15]. The thrombotic response to an induced vascular injury was much stronger with antiprothrombin than with control antibody. However, the antibodies used in these studies were not well characterized. In rare cases antiprothrombin antibodies can cause bleeding due to decreased levels of prothrombin because, in contrast to autoantibodies against β_2GPI, antiprothrombin antibodies enhance clearance of prothrombin from the circulation [16].

Annexin A2

The annexins constitute a family of highly conserved, Ca^{2+}-regulated, phospholipid-binding proteins that have many functions related to membrane-mediated processes [17]. Annexin A2 influences haemostasis as it is a receptor for tissue plasminogen activator (tPA) on endothelial cells and for β_2GPI. Annexin A2 knockout (−/−) mice show deposition of fibrin in the lungs, spleen, liver, and kidney as they age consistent with decreased fibrinolysis [18]. Annexin A2 autoantibodies develop in patients with APS; high titers of anti-annexin A2 autoantibodies correlate with thrombosis [19]. Annexin A2 (−/−) mice, in a model of APS, reduce the prothrombotic effect of injected aPL, suggesting that the mechanism by which annexin A2 is involved in APS is due to disruption of its fibrinolytic function by the autoantibodies [20].

Annexin A5

Annexin A5 is another member of the annexin family of calcium-dependent phospholipid-binding proteins. The anticoagulant properties of this protein result from its rapidly forming two-dimensional crystal arrays over the polar heads of the anionically charged membrane phospholipids [21]. Anionic phospholipids are required cofactors for the four critical phospholipid-dependent coagulation reactions: the IXa-mediated tenase reaction, the tissue factor-VIIa-mediated tenase, the tissue factor-VIIa-mediated IXase reactions, and the prothrombinase reaction. Assembly of the annexin A5 array shields phospholipids from contributing to the enzymatic reactions. Annexin A5 knockout mice have increased placental thrombosis and infarction but no increased propensity for systemic thrombosis [22].

Several studies explore the possibility that anti-annexin A5 antibodies correlate with clinical manifestations of APS [23]. IgG anti-annexin A5 antibodies occur in patients with pregnancy complications but not in those with venous or arterial thrombosis.

An alternative research path asks whether aPL antibody-mediated disruption of annexin A5 crystallization, on activated platelets and on phospholipid vesicles, leading to reduction of anticoagulant activity (called "A5 resistance") correlates with adverse clinical outcomes. Recent data suggest that A5 resistance does correlate with increased risk of thrombosis and pregnancy complications. A recent paper [24] correlates A5 resistance with increased prevalence of thrombosis in a "real-world" retrospective population and in a group of prospectively observed asymptomatic patients. In both the retrospective and prospective groups, A5 resistance correlates with positivity for multiple criteria-based aPL assays.

In summary, A5 resistance correlates with an increased risk for thrombosis. The resistance is specifically mediated by anti-domain one of β_2GPI [25] and potentially other aPL cofactor proteins, but it is not mediated by antibodies to annexin A5. To date, although anti-annexin A5 antibody assays may be associated with an APS process, it is not clear that they have a causal relationship to the disease.

Platelet Factor 4 (CXCL4) and Other Platelet-Derived Chemokines

Platelet factor 4 (PF4) or CXCL4 is an 8kD molecule belonging to the CXC chemokine family. It circulates as a tetramer. It was first recognized to play a role in APS when platelet membrane protein extracts, from three healthy donors and seven APS patients, were passed through a β_2GPI affinity column and analysed by mass spectrometry [26]. Experiments using in silico molecular docking models indicated that a tetramer of PF4 act as a scaffold to which two molecules of β_2GPI bound. According to this model, domain I of β_2GPI became accessible for recognition by aβ_2GPI, while domain V was available to interact with other proteins on the platelet membrane [26]. The multimeric

complex $(PF4)_4/(\beta_2GPI)_2/a\beta_2GPI$ can exist in solution. Furthermore, platelets from healthy individuals, primed with very small amounts of thrombin, were activated only when PF4, β_2GPI and $a\beta_2$GPI were present and were associated with the phosphorylation of p38 MAP kinase. Natural dimerization of β_2GPI is necessary for more effective recognition by $a\beta_2$GPI, the whole complex being a powerful platelet activator [27]. The interaction of β_2GPI with PF4 induces β_2GPI dimers in a completely natural way and facilitates antibody binding and platelet activation, which itself is important for enhanced activation of endothelium and fibrinogen in a mouse thrombosis model. Plasma levels of platelet-derived chemokines such as PF4, PF4var (PF4 variant, also known as CXCL4L1), CXCL7, and CCL5 are elevated in patients with APS but not in patients with systemic lupus erythematosus (SLE), coronary artery disease (CAD), or healthy controls [28], indicating marked platelet activation in APS patients. These data support the notions that platelet activation in APS is induced by the complex $(PF4)_4/(\beta_2GPI)_2/a\beta_2GPI$.

Other Proteins

A recent study suggests that true anticardiolipin antibodies (those that recognize cardiolipin without the support of a plasma protein) may also induce a prothrombotic state in mice [28]. It is difficult to prove that these antibodies function in the absence of a natural protein because, in in vivo models, many candidate molecules are obligatorily present. Anticardiolipin antibodies, as measured with currently available assays, correlate weakly with thrombosis compared to lupus anticoagulant (LA); moreover, syphilis and leprosy patients have these autoantibodies without a clear increased risk of thrombosis. Cardiolipin is likely too small to elicit an immune response on its own without a carrier protein.

Autoantibodies to a number of other coagulation-relevant proteins, such as protein S, protein C, tissue factor pathway inhibitor, factor X, XI and XII, are found in a small subgroup of patients with APS [29]. Some correlate with clinical manifestations, and mechanisms regarding how these autoantibodies might induce a prothrombotic state have been proposed, but none of the autoantibodies have been tested in in vivo models. There is no convincing evidence that they play a role in thrombosis or pregnancy complications in APS.

Why Do Plasma Proteins Become Prothrombotic in the Presence of Autoantibodies?

Based on animal models of APS, three natural proteins, β_2GPI, prothrombin, and annexin A2, are identified as important antigens in APS. Although inhibition of annexin A2 can inhibit fibrinolysis, the absence of plasminogen does not cause high risk for thrombosis in humans, suggesting a minor role of annexin A2.

Autoantibodies against prothrombin differ from autoantibodies against a prothrombin- phosphatidylserine (PS) complex. There are autoantibodies that recognize prothrombin only when it is bound to an anionic phospholipid, suggesting that prothrombin undergoes a conformational change when it is bound to PS, exposing a cryptic epitope. Based on human studies, antibodies directed against this cryptic epitope correlates better with thrombosis than do antibodies against the rest of the prothrombin molecule. Further analysis of the specific antigenic epitope will help us understand how these autoantibodies might be prothrombotic.

It is not immediately clear why antibodies against β_2GPI or prothrombin could induce thrombosis. Inhibition of prothrombin would result in bleeding, and no physiological function has been described for β_2GPI to explain its role in a prothrombotic risk. To become prothrombotic, the autoantibodies should induce a new property in their target proteins. Here are a few possible mechanisms: (a) increased affinity due to dimerization by antibodies [27], (b) conformational changes and expression of a hidden epitopes [30], and (c) reshuffling of disulphide bridges within proteins [31].

Conformational Changes of β_2-Glycoprotein I

The first demonstration that a plasma cofactor was required for aPL to bind cardiolipin was made by McNeil et al. in 1989 [32]. In the following year, this cofactor was identified as β_2GPI by peptide sequencing which itself was later identified as the major autoantigen for aPL [33, 34]. β_2GPI consists of 326 amino acid residues organized in five CCP (complement control protein) domains [35] (DI-DV), which function as protein-protein interaction modules in many proteins. DI-DIV have evolutionary conserved sequences; DV contains a six-residue insertion, a 19-residue C-terminal extension and an additional disulphide bond that includes a C-terminal cysteine residue. DV also harbours a large, positively charged patch that determines affinity for anionic phospholipids. The crystal structure of β_2GPI, solved in 1999, [36, 37] suggests a stretched arrangement of the DI-IV, with DV lying at a right angle to the other domains, in the shape of a J. The phospholipid-binding site is located at the bottom of DV and consists of 14 charged amino acid residues and a flexible and hydrophobic loop. This crystal structure predicts that, when β_2GPI is bound to a lipid layer, DI to IV point away from the lipid layer and that the potential binding site for aβ_2GPI autoantibodies in DI is fully exposed [38].

There are no circulating nor tissue deposition of β_2GPI-antibody immune complexes in patients with APS. A logic interpretation of this observation is that the antibodies directed against β_2GPI do not recognize β_2GPI in the circulation. The antibodies recognize a cryptic epitope in the molecule. It has been shown that β_2GPI expose the autoantibody binding site when it binds to anionic phospholipids [39].

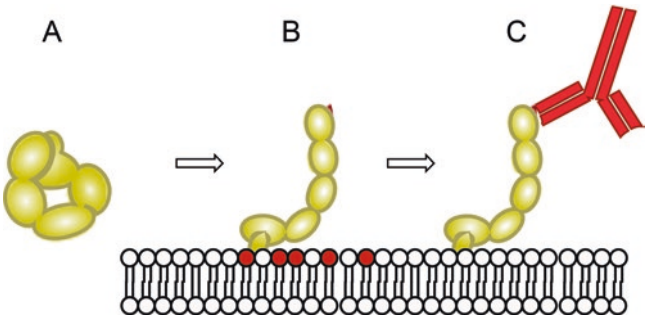

Fig. 2.1 Conformational change within β_2GPI. (*A*) β_2GPI as it circulates in plasma. (*B*) Binding to negatively charged phospholipids opens up β_2GPI. (*C*) Binding of autoantibodies stabilizes β_2GPI in its stretched conformation

Electron microscopy studies show that β_2GPI exists in two different conformations (Fig. 2.1). In plasma, it is present as a circular protein in which DI interacts with DV. On binding to anionic surfaces, the protein opens up and expresses the #hockey-stick conformation of the crystal structure [40]. The circular conformation predicts shielded epitopes within DI and DV [40], and, indeed, autoantibodies against DI recognize β_2GPI only when it is bound to anionic surfaces, not when it is present in the circulation. Since β_2GPI binds to cell receptors via its DV, and since binding is enhanced by autoantibodies, it must be mediated by a cryptic epitope in DV that is expressed after the molecule has opened.

Small-angle X-ray scattering (SAXS) experiments suggested that in solution, β_2GPI adopts an S-shaped conformation with an additional buckle between DII and DIII [41]. Additional SAXS experiments show that β_2GPI adopts different conformations, depending on pH, ionic strength, and certain cations. β_2GPI turns out to be a flexible molecule, not constrained to a single, specific conformation; its conformation depends on interactions with its surroundings. Apparently β_2GPI can adapt a number of different structural conformations that in vitro can coexist in a dynamic equilibrium. Factor H, a complement factor built up of 20 CCP domains, also adopts different domain orientations in solution with consequences for its functional activity [42, 43]. Proteins consisting of CCP domains vary their conformations, depending not only on the length and flexibility of the linker sequences between domains but also, predominantly, on interactions with their surroundings.

Redox Balance

Antiphospholipid syndrome is characterized by oxidative stress and systemic inflammation [44, 45]. The overproduction of reactive oxygen species (ROS) results in an oxidative microenvironment that exacerbates inflammation, inducing cell death and tissue damage, compromising antioxidant defence mechanisms [46]. Patients with APS have high levels of circulating pro-inflammatory cytokines interleukin-2 (IL-2), interleukin-6 (IL-6), and tumour necrosis factor (TNF), together with markers of oxidative stress and inflammation such as serum amyloid A (SAA), C-reactive protein (CRP), 8-isoprostane, and prostaglandin E2 (PGE2) [47, 48].

In vivo and under normal physiological conditions, β_2GPI is produced in the liver and exists predominately in circulation in its free thiol form, which is less immunogenic than the oxidized form (Fig. 2.2). The precise role of β_2GPI and its different forms are complex [49]; it is thought to act as a natural anticoagulant that mediates a range of functions including the clearance of liposomes, apoptotic bodies and microparticles [49–52].

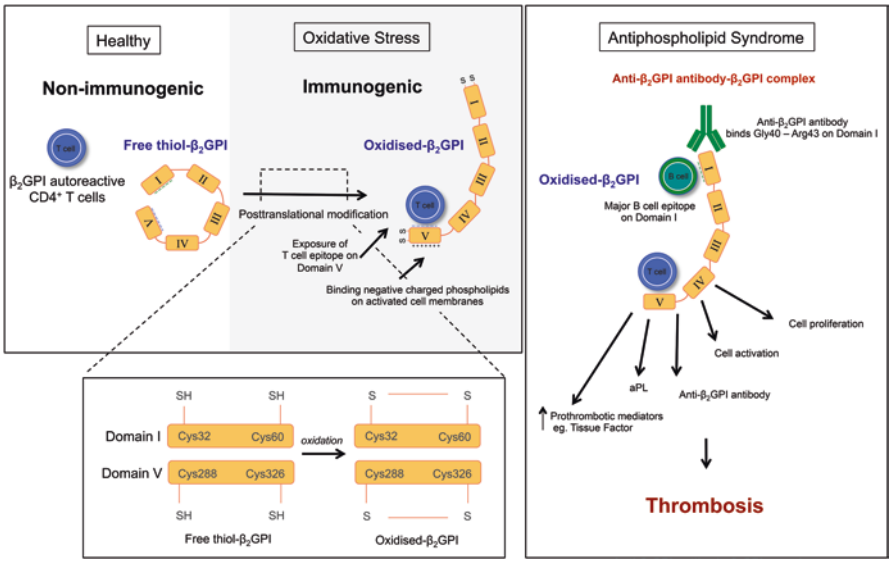

Fig. 2.2 How does oxidized β_2GPI participate in the formation of thrombotic APS? During oxidative stress, free thiol β_2GPI can undergo post-translational modification to form the immunogenic form, oxidized β_2GPI after binding phospholipids. β_2GPI autoreactive CD4$^+$ T cells recognize newly exposed epitopes located on Domain V but not on free thiol β_2GPI. A complex is formed between aβ_2GPI, autoreactive CD4$^+$ T cells and oxidized β_2GPI triggering the production of aPL, specifically aβ_2GPI, cell proliferation and the release of pro-inflammatory cytokines which are key events in the pathophysiology of thrombotic APS

Quantitation of Oxidized β$_2$GPI as a Biomarker for Antiphospholipid Syndrome

Oxidized β$_2$GPI level in APS has been proposed as a biomarker of thrombotic risk for APS. Levels of oxidized β$_2$GPI in patients with APS are higher than in healthy subjects and patients with other autoimmune disease or non-aPL disease controls with thrombosis [53]. Free thiol β$_2$GPI may play a protective role in APS, since free thiol β$_2$GPI protects human umbilical vein endothelial cells (HUVEC) against hydrogen peroxide-induced cell injury [54]. Decreased plasma free thiol β$_2$GPI may thus lower the physiological buffer against oxidative stress-induced injury. Free thiol β$_2$GPI also protects human retinal pigment epithelium and the subretinal endothelial cell against oxidative, hydrogen peroxide stress-induced, cell death [55].

A multicentre, cross-sectional, international study using prospectively acquired samples has demonstrated that the redox status of β$_2$GPI differs between healthy individuals and patients with thrombotic APS [53]. In the former it exists predominately with free thiols; APS patients have higher levels of total and oxidized β$_2$GPI compared to both healthy subjects and patients with other autoimmune disease [53, 56].

Diagnostic and Prognostic Implications of the Oxidized β$_2$GPI ELISA

Anticardiolipin antibodies, aβ$_2$GPI, and LA test serve as diagnostic markers in APS. The predominant isotypes of aPL in APS patients are IgG aCL and IgG aβ$_2$GPI [57, 58]. Although, non-criteria or non-classical aPL such as antiphosphatidylserine, antiphosphatidylethanolamine, and antiphosphatidylglycerol have been reported, only the three classical aPL tests are used in diagnosis of APS [59]. The LA assay identifies autoantibodies against either prothrombin and/or β$_2$GPI, whereas the aCL assay detects the aCL and/or aβ$_2$GPI antibodies. The aβ$_2$GPI assay detects only antibodies against β$_2$GPI. Concomitant triple positivity for aCL, β$_2$GPI and LA may indicate severe APS and high recurrence risk [60], a point that is still controversial [61]. Lupus anticoagulant correlates much better with the clinical manifestations of APS than the detection of the autoantibodies with an ELISA [62, 63], and a positive LA assay due to aβ$_2$GPI has a stronger correlation for thrombotic risk than due to antiprothrombin autoantibodies [64].

In a clinical setting, it is important to stratify risk for development of clinical events in APS and in asymptomatic, aPL-positive individuals. Delayed or inadequate treatment can result in damage and impaired quality of life [65, 66]. Although β$_2$GPI levels are not routinely measured in patients with APS, considering the specificity of high levels of oxidized β$_2$GPI, measuring their levels may assist in diagnosis and prognosis. The level of oxidized β$_2$GPI is calculated by subtracting the concentration of free thiol from total β$_2$GPI. Using an ELISA to measure post-translational redox modifications of β$_2$GPI (including total and free thiol β$_2$GPI) [53] (Fig. 2.3) and β$_2$GPI

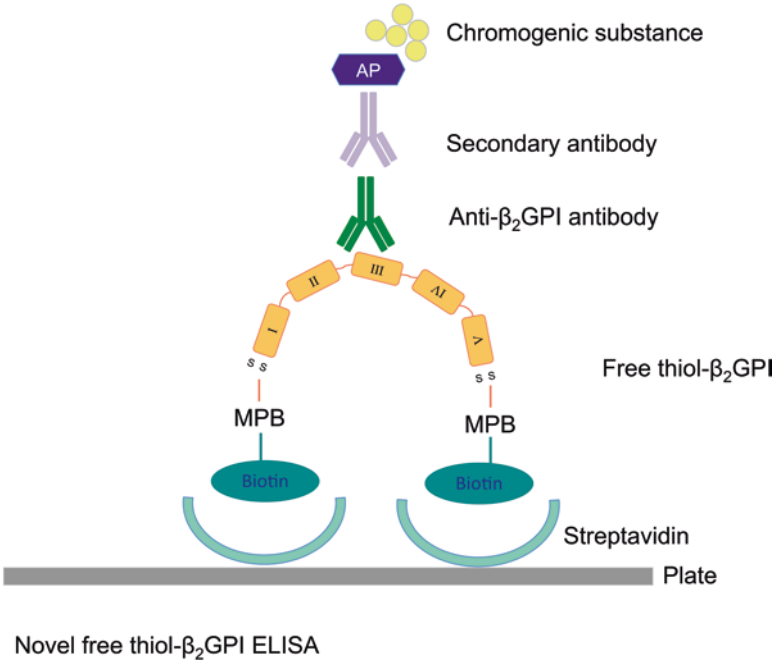

Fig. 2.3 Schema of enzyme-linked immunosorbent assay (*ELISA*) to measure free thiol β_2 glycoprotein I (β_2GPI). Free thiol β_2GPI bind streptavidin via biotin-conjugated maleimidyl-propionyl biocytin (*MPB*) and become immobilized. Acetone precipitation removes unbound MPB, and bound free thiol β_2GPI is quantified with aβ_2GPI and detected using a secondary antibody, alkaline phosphatase (*AP*) and chromogenic substance para-nitrophenylphosphate (PnPP)

plasma levels in 359 patients (identified through an international multicentre initiative) who had either APS or other autoimmune diseases or non-APS vascular thrombosis, Ioannou et al. found that the redox state of β_2GPI and its concentration in APS patients had a profile distinct from that in the various control groups.

Group Conclusion

Evidence from both clinical and animal studies supports the concept that β_2GPI is the main autoantigen in APS. Understanding of the pathophysiology of APS and the involvement of β_2GPI and its post-translational modified forms remains incomplete; understanding the relevance of oxidized β_2GPI in APS will be important. Although #aPL are a defining, hallmark feature of APS, their presence does not exclusively indicate APS nor do they stratify individuals for risk of thrombosis.

Current methods for diagnosing APS patients do not incorporate quantitation of total and free thiol β_2GPI. Specific ELISAs for quantifying these parameters may enhance our diagnostic and prognostic capabilities. Prospective studies may validate

measurement of oxidatively modified forms of β_2GPI as biomarkers. The AntiPhospholipid Syndrome Alliance For Clinical Trials and InternatiOnal Networking (APS ACTION), an international research network that collects patient samples from 25 centres around the world [67], may allow a longitudinal study that measures oxidized β_2GPI.

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Chapter 3
Origin of Antiphospholipid Antibodies

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Introduction

The antiphospholipid syndrome (APS) is a multisystem syndrome characterized by thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL) [1–5]. These antibodies are directed against phospholipid-binding proteins (described in Chap. 2), such as β_2-glycoprotein I (β_2GPI), and may be either nonpathogenic or pathogenic. Replacing the heavy chain of a nonpathogenic anti-β_2GPI antibody (aβ_2GPI) with the heavy chain from a pathogenic aβ_2GPI renders that antibody capable of inducing experimental APS [6].

Infectious agents are among the main triggers for the production of aβ_2GPI. Molecular mimicry between β_2GPI-derived synthetic peptides and structures within bacteria, viruses, tetanus toxoid, and cytomegalovirus results in the induction of experimental APS [7–10]. Other potential triggers for aPL production include the microbiome and cell death. Innate immune activation is a common feature that likely serves as a second hit for aPL induction, whether from the initiating trigger itself or as a second stimulus. Apparently healthy individuals have the potential to produce aPL, which, particularly when of the IgM subclass, can be protective [11, 12]. In contrast, in a pro-inflammatory microenvironment such as that triggered by infection, injury, or commensal microorganisms, and on the appropriate genetic background, pathogenic aPL may emerge. This chapter outlines the environmental and immune factors leading to aPL and APS; genetic factors are discussed in Chap. 4.

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What Is Known?

Infections

The Association Between Infections and Antiphospholipid Antibodies

During the last decade, common bacteria and viruses and vaccines have been associated with induction of APS [7–10, 13–15]. Many infections are accompanied by the appearance of aPL that, in some cases, are associated with clinical manifestations of APS (reviewed in [13–15]). Skin infections (18%), human immunodeficiency virus (HIV) (17%), pneumonia (14%), hepatitis C virus (HCV) (13%), and urinary tract infections constitute the most common infections found to trigger aPL and APS. In many cases, more than one agent/organism was identified as the source of infection. Other infections less frequently associated with APS include *Mycobacterium leprae* (*M. leprae*) (leprosy), *Spirillum minus* (rat-bite fever), *Treponema carateum* (pinta), *Pneumocystis carinii*, mycoplasma, pulmonary tuberculosis, malaria, and leptospirosis. Recently, catastrophic APS (CAPS) was associated with H1N1 influenza infection [15].

Catastrophic APS is an unusual and a potentially fatal variant of the APS [16]. Since its initial definition, more than 500 patients have been described [4, 16–20]. "Triggering" factors, increasingly apparent, were present in 51% of reported cases. These include trauma (for instance, surgical), anticoagulation withdrawal, carcinomas, and infections (identified in 24% of patients, including respiratory, cutaneous, urinary tract, gastrointestinal, and sepsis). Molecular mimicry between the infectious pathogen and a "self-antigen" has been proposed as a major mechanism responsible for development of CAPS following viral, bacterial, or parasitic infections, but the rapid onset of CAPS suggests that other mechanisms (e.g., NETosis or superantigen responses) may be involved. The main infectious agents associated with the development of aβ_2GPI and APS are summarized in Table 3.1.

Table 3.1 Infectious agents associated with anti-β_2GPI and antiphospholipid syndrome

Infectious agent	APS manifestations
Staphylococcus aureus	CAPS [142]
Streptococcus pyogenes	Cardiac valve and CNS lesions [133, 137]
Escherichia coli	CAPS [143]
Klebsiella	CAPS [144]
Hepatitis C	Thrombosis, brain infarction [145]
Epstein-Barr virus	PE, thrombosis [146]
Parvovirus B19	Thrombosis [147]
Cytomegalovirus	Thrombosis, stroke [9, 148]
HIV	Leg ulcer necrosis, arterial and venous thrombosis, vasculitis, livedo reticularis [149]
HSV	CAPS [150]

CAPS catastrophic antiphospholipid syndrome, *CNS* central nervous system

The Molecular Mimicry Hypothesis

Blank et al. [21, 22] hypothesized that molecular mimicry between infectious pathogens and β_2GPI may serve as the origin of aPL and APS. Their theory was based on two lines of evidence: the striking association between APS and infectious agents and a strong amino acid similarity between β_2GPI-derived peptides and various common pathogens (Table 3.2). Using a hexapeptide phage display library, they [21] identified several synthetic peptides, which exhibited high homology with proteins from the membrane particles of different bacteria and viruses, as target epitopes for monoclonal aβ_2GPI derived from APS patients. For example, the sequence LKTPRV showed homology to eight different bacteria, such as *Pseudomonas aeruginosa*, and five kinds of viruses, such as human cytomegalovirus, while the sequence TLRVYK had homology to other bacteria and viruses. Furthermore, by neutralizing pathogenic aβ_2GPI, these peptides inhibited both endothelial cell activation in vitro and induction of experimental APS in vivo.

To demonstrate that molecular mimicry can trigger experimental APS, Blank and coworkers [7] immunized naïve mice with microbial pathogens that share structural homology with the TLRVYK hexapeptide. All immunized mice developed

Table 3.2 Candidate peptides with structural and functional similarity to the phospholipid-binding region of Domain V of β_2GPI

Peptide	Source	Amino acid sequence	Inhibition of β_2GPI binding to cardiolipin (%)[a]
GDKV	Gly_{274}-Cys_{288} in Domain V of human B2GPI	GDKVSFFCKNKKC	43
$GDKV_2$	Modified GDKV with all six residues between Lys_{282} and Lys_{287} replaced with Lys	GDKVSFFCKKKKKKC	56
TADL	Thr_{77}-Glu_{96} of Adv type2 DNA-binding protein	TADLAIASKKKKKRPSPKPE	68
TIFI	Thr_{101}-Thr_{120} of ULB0-HCMVA from human CMV	TIFILFCCSKEKRKKKQAAT	75
VITT	Val51-Ile70 of US27-HCMVA from human CMV	VITTILYYRRKKKSPSDT	83
SGDF	Ser_{237}-Ser_{256} of TLP-BACSU from *Bacillus subtilis*	SGDFEYTYKGKKKKMAFATS	N/A

PL phospholipid, *CMV* cytomegalovirus, *N/A* not available

[a]Refers to the percentage of inhibition of 100 nM of β_2GPI binding to cardiolipin produced by 6 μM of each peptide

anticardiolipin antibodies (aCL) and aβ_2GPI; the highest levels of aβ_2GPI were found in mice immunized with *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Candida albicans*, or tetanus toxoid, while the lowest aβ_2GPI levels were found in mice immunized with *Klebsiella pneumoniae*. Passive transfer of anti-TLRVYK antibodies (from immunized mice) into naïve mice resulted in thrombocytopenia, lupus anticoagulant (LA) activity, and increased fetal loss, i.e., experimental APS similar to mice injected with a pathogenic monoclonal aβ_2GPI [7]. These findings demonstrate that bacteria with protein sequences homologous to β_2GPI can induce aβ_2GPI and APS manifestations [7], and provide evidence for a role for molecular mimicry in experimental APS.

Gharavi et al. [23] induced circulating aβ_2GPI into naïve mice by immunizing with synthetic peptides derived from bacterial or viral proteins that show sequence similarity with a 15 amino acid peptide (GDKV) in the phospholipid-binding domain (Domain V) of β_2GPI. The synthetic peptides included regions from the proteins of human adenovirus, cytomegalovirus, and *Bacillus subtilis*. Mice immunized with the peptides produced high levels of aCL and aβ_2GPI, suggesting that viral and bacterial proteins may function like β_2GPI and produce aPL through molecular mimicry of β_2GPI. β_2-glycoprotein I-derived synthetic peptides from regions other than Domain V, such as peptide NTLKTPRV from Domain I [21], also share sequence similarities with common bacterial antigens and interact specifically with aβ_2GPI in mice, decreasing its thrombogenic potential [24]. Finally, the relationship between pathogens and β_2GPI extends beyond sequence homology. β_2-glycoprotein I binds to bacterial lipopolysaccharide (LPS), which is recognized by toll-like receptor 4 (TLR4) [25, 26], and functions as an in vivo scavenger of LPS [25]. The peptide sequence in Domain V responsible for LPS binding is conserved in all mammals [25]. An association between TLR4 gene polymorphisms and APS has been reported [27].

Leprosy and Syphilis

The association of infection with aPL and APS has been most thoroughly investigated in two infectious diseases, leprosy and syphilis. Studies in these infections highlight the need for astute differential diagnosis and careful characterization of the observed aPL in determining the pathogenic role of infection-induced aPL.

Leprosy (*M. leprae*)

Clinical presentation of leprosy Leprosy, which has a wide range of clinical presentations, is caused by infection with the acid-fast bacillus *M. leprae*. The most basic classification separates leprosy into “paucibacillary” and “multibacillary” forms [28]. Multibacillary leprosy patients have acid-fast bacilli visible on bacilloscopic studies, high anti-*M. leprae* antibody titers, and more disseminated disease.

Paucibacillary leprosy patients have no acid-fast bacilli visible on bacilloscopic studies and are treated with a shorter course of anti-*M. leprae* antibiotics. Leprosy can be subdivided into five forms based on clinical and histopathologic findings: tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), and lepromatous (LL) [29]. Individuals with BT, BB, and BL leprosy are at risk for a Type 1 reaction, an inflammatory response thought to relate to *M. leprae* antigen release upon introduction of anti-leprosy therapy [30]. In contrast, BL and LL leprosy are associated with a Type 2 reaction, called erythema nodosum leprosum (ENL), thought to relate to immune complex formation in the setting of high antibody and high *M. leprae* antigenic load [31].

Antiphospholipid antibody prevalence in leprosy Ribeiro et al. [32] found that 49% of 158 leprosy patients were positive for any aPL (46.2% for aβ_2GPI and 15.8% for aCL), with IgM being the predominant isotype (88% and 84%, respectively). Compared with primary APS patients, leprosy patients had a higher prevalence of aβ_2GPI (46.2% [73/158] in leprosy versus 23.7% [9/38] in APS) and lower prevalence of aCL (15.8% [25/158] in leprosy versus 89.5% [34/38] in APS). The frequencies of aβ_2GPI and aCL were the same in leprosy patients who had completed or were still receiving anti-leprosy therapy; the frequencies were not increased in patients with leprosy immune reactions. Ribeiro et al. [33] followed aPL titers in 37 leprosy patients for a mean follow-up of 66.8 months. Thirty-two (86%) remained positive: 84% for aβ_2GPI and 19% for aCL. Antiphospholipid antibody prevalence was also high (78%) in a study of 51 LL and BB leprosy patients from Argentina without clinical APS [34]. The rates of seropositivity for specific aPL were 57% (aβ_2GPI), 61% (aCL), and 69% (LA), mostly IgM. The rate of aPL positivity did not differ during or following treatment [34]. Leprosy patients with aPL had higher plasma levels of soluble adhesion molecules such as P-selectin than did patients without aPL. The authors postulate that this finding relates to aPL-mediated activation of vascular endothelium [35].

Some studies have reported much lower rates of aPL: 3% aβ_2GPI and 37% aCL positivity in a cohort of 35 multibacillary leprosy patients from Egypt [36]. Because these patients had leprosy for 15.2 ± 9.2 years, it is possible that their leprosy and any associated reactions (including aPL) would have resolved. No APS or thromboembolic phenomena were reported in these patients. As antibody levels and immune complex levels decrease as bacterial burden decreases, it is important to consider the stage of the disease when assessing aPL and APS. To evaluate patients prior to anti-leprosy therapy, Baeza et al. [37] studied 30 untreated multibacillary LL patients, of whom 23 (77%) were positive for IgG aCL and 23 (77%) were positive for IgM aCL. Additionally, 25 (83%) of LL sera bound to non-bilayer lipid arrangements containing mycolic acid. Levels of aCL IgG and IgM correlated with antibody reactivity to non-bilayer phospholipid ($r = 0.77$ and $r = 0.69$, respectively, $p < 0.0001$). The authors hypothesize that antibodies to non-bilayer phospholipid may disrupt cellular membranes, leading to the release of potentially immunogenic cellular components, such as aCL.

Antiphospholipid antibodies and the risk of thrombosis in leprosy The association of aPL with thrombosis in leprosy is unclear. The fact that leprosy and systemic lupus erythematosus (SLE) have similar clinical and laboratory findings may make differential diagnosis difficult [38]. One study [39] found that 16% of 100 patients with multibacillary leprosy had four or more diagnostic criteria for SLE, yielding an 84% specificity for the diagnostic criteria. Overall, 20% of the 100 patients had one or more detectable aPL (aCL IgG, aCL IgM, LA, or Venereal Disease Research Laboratory [VDRL] test). However, none of the 20 patients with aPL had a history of vascular thrombosis or pregnancy loss. In a study that included seven multibacillary leprosy patients with a history of APS, only two had elevated aβ_2GPI (2 IgM, 1 IgG) after the thrombotic event [40]. A case report described a BT leprosy patient who developed bilateral toe gangrene [41]. The patient was positive for aCL IgM at presentation and after 6 weeks of therapy; however, ultrasound was negative for arterial and venous thrombi.

Deep vein thrombosis (DVT) is an emerging risk in patients with multibacillary leprosy who receive thalidomide and corticosteroids for ENL [42–46]. The typical sequence of events preceding DVT is treatment with multi-antibiotic therapy for *M. leprae*, development of ENL, initiation of treatment with a corticosteroid, addition of thalidomide with corticosteroid taper, and development of DVT during the cross-taper of thalidomide and corticosteroid. In one such case, aβ_2GPI and aCL IgG were slightly elevated when the patient developed DVT after 2 months of thalidomide treatment. The antibody levels returned to normal 8 weeks after the DVT [42].

Lucio's phenomenon, characterized by recurrent ulcerative lesions affecting mainly the lower extremities, is a severe and potentially fatal immune reaction that occurs in patients with LL. In one case report from Ecuador of Lucio's phenomenon with APS, aCL IgM was positive, and dermal vessels were occluded by thrombi [47]. Other reports found aCL positivity with vasculitis, not thrombosis [48, 49]. Some reports lack aPL testing but suggest evidence of thrombosis [50]. Levy et al. [51] found that aCL were β_2GPI dependent in only two of 33 (6%) individuals with leprosy, both of whom had Lucio's phenomenon. Forastiero et al. [52] compared the thrombogenic properties of IgM antibodies isolated from patients with leprosy or APS (and high levels of aCL, aβ_2GPI, and LA) in a murine model of thrombosis. They found that IgM aPL from leprosy patients did not have thrombogenic and pro-inflammatory effects in vivo, when compared to aPL from APS patients, and present this as data supporting their hypothesis that thrombosis risk may relate to aPL type.

A study using mutant β_2GPI showed that APS-derived aPL bound better to Domain V-deleted than to Domain I-deleted β_2GPI, whereas leprosy-derived aPL bound to both mutant forms [53]. Furthermore, an anti-Domain I monoclonal antibody inhibited binding of APS-derived, but not leprosy-derived, aPL to β_2GPI [53]. This difference in binding specificity may explain, at least in part, the different thrombogenic potentials of APS- versus leprosy-derived aPL.

Antiphospholipid antibodies and genetic polymorphisms in the β_2GPI-encoding apolipoprotein H (APOH) gene A number of groups have examined whether genetic polymorphisms in the *APOH* gene (chromosome 17) that encodes β_2GPI

differ in leprosy patients that develop aPL and thrombosis. Brochado et al. [40] characterized four single nucleotide polymorphisms (SNPs) in *APOH* in a cohort of 117 leprosy patients (seven had a history of APS, as defined by aPL positivity and confirmed thrombosis) and 113 non-leprosy controls in Brazil. They reported increased Leu/Leu and Val/Val at the Leu247Val SNP site (rs4581) in the leprosy group. Moreover, the multibacillary leprosy group with positive aβ_2GPI IgM had an increased frequency of Val/Val homozygosity compared to controls [40]. The frequency of the mutant allele Ser316 was also higher in this group. In contrast, the two other SNPs examined, Cys306Gly and Trp316Ser, did not show significantly different allelic frequencies in leprosy patients compared with controls. Interestingly, the allele frequency at Leu247Val did not vary in a cohort study of Polish individuals with or without APS, and there was no association of the Leu247Val genotype with aβ_2GPI levels [54].

Syphilis (Spirochete Treponema Pallidum)

Serological evaluation of syphilis Syphilis is caused by infection with the spirochete *Treponema pallidum*. The serologic diagnosis of syphilis is reviewed by Morshed and Singh [55]. Non-treponemal tests for syphilis detect antibodies produced by the host in response to damaged cells. One of these antibodies is directed to cardiolipin, which is released by both damaged human cells and spirochetes. These tests have a high false-positive rate, given the nonspecific nature of these antibodies. For this reason, treponemal tests are also included in the diagnostic evaluation for syphilis.

Antiphospholipid antibody prevalence in syphilis In a study on aPL and infectious diseases in South Africa, the rates of aCL and aβ_2GPI positivity in syphilis were lower than in leprosy (8% versus 29% and 28% versus 89%, respectively) [56]. However, stage of disease, concurrent medications, and other information were not detailed. A study of aPL in Brazil included 74 syphilis patients who were positive in both VDRL and fluorescent treponemal antibody (FTA) tests, with most having completed penicillin treatment [57]. The rate of aPL positivity in these syphilis patients was 18% aCL IgG, 13% aCL IgM, and 10% aβ_2GPI. No thrombotic events were noted. Guerin et al. [58] reported on aβ_2GPI incidence among patients with a positive VDRL but negative confirmatory test for syphilis; these "false-positive" patients had detectable aβ_2GPI IgM (17%), IgG (17%), and IgA (33%).

Antiphospholipid antibodies and the risk of thrombosis in syphilis Affinity-purified aCL from five syphilis patients did not have LA activity, and only one inhibited prothrombin-to-thrombin conversion in vitro [59]; in contrast, all APS-derived aCL had both activities. In phospholipid-binding assays, syphilis-derived aCL recognized cardiolipin, but not phosphatidylserine, while all APS-derived aCL recognized both phospholipids. The dependence of phospholipid binding on β_2GPI was not indicated in this study. The authors suggest that the difference in phospholipid recognition explains why hypercoagulability is not observed in patients with syphilis. Early studies demonstrated that APS-, but not syphilis-, derived aCL requires β_2GPI for binding

to phospholipid [60, 61], but recent literature suggests that the distinction between "autoimmune" and "infectious" aPL is less absolute than previously believed [62]. Infection could trigger induction of pathogenic aPL in genetically predisposed individuals, and the resulting aPL may be heterogeneous in their dependency on β_2GPI.

Binding affinity and antibody isotype also play a role in aPL/β_2GPI interactions. Metzger et al. [63] found that sera from syphilis patients bind to β_2GPI under low salt conditions, but not higher (300 mM) salt conditions, in which APS sera still show strong binding, indicating that the binding affinity of aβ_2GPI in patients with syphilis is lower than that in APS patients. Together, the differences in aPL derived from syphilis versus APS patients support the low incidence of aPL-associated thrombosis in syphilis patients.

The Microbiome

The chronic triggers that sustain aPL in APS patients are elusive. Emerging data suggest that microbiota, commensal organisms that colonize human hosts, likely contribute to APS pathogenesis [64]. "Commensalism" is a symbiotic relationship between two organisms of different species in which one derives some benefit, while the other is unaffected. The microbiota colonizes every niche of the human body, including the oral mucosa, gastrointestinal tract, sinobronchopulmonary (respiratory) tract, skin, and urogenital tract [65]. The barrier organ with the largest diversity of microbiota is the gut, which will be the focus of this section; other niches may also harbor triggers of aPL.

Gut commensals influence many aspects of innate and adaptive immunity [66]. Most notably, commensals induce key murine CD4 helper T cell subsets implicated in autoimmunity [67]. Segmented filamentous bacteria, species that colonize the murine small intestine by firmly attaching to the epithelium, are capable of inducing Th17 cells that specifically recognize segmented filamentous bacteria antigens [68]. Colonization with human colonic *Clostridia* species (Cluster IX and XIVa) leads to differentiation of regulatory T cells (Treg) in gnotobiotic mice (germ-free animals colonized with a defined set of microbes) [69]. Dysregulation in both Treg and Th17 cells is a well-established cellular mediator of autoimmunity. Follicular helper T cells (Tfh), a CD4 T cell subset that supports B cell antibody production, are key promotors of humoral immunity and autoantibody formation [70]. Murine Tfh are dependent on the gut microbiota [71], and B cell development occurs not only in the bone marrow but also in the gastrointestinal tract of mice [72].

The gut microbiome, extensively characterized in health and in several immune-mediated diseases [65, 73], is currently under investigation in APS patients [74]. While causal relationships between the microbiome and autoimmune diseases are difficult to establish in human studies, progress has been made in animal models. Several murine models of autoimmune disease are modulated by gut microbiota [67]. These microbiota-related effects are best demonstrated through germ-free rederivation of such models. The most dramatic examples are the loss of spondylo-

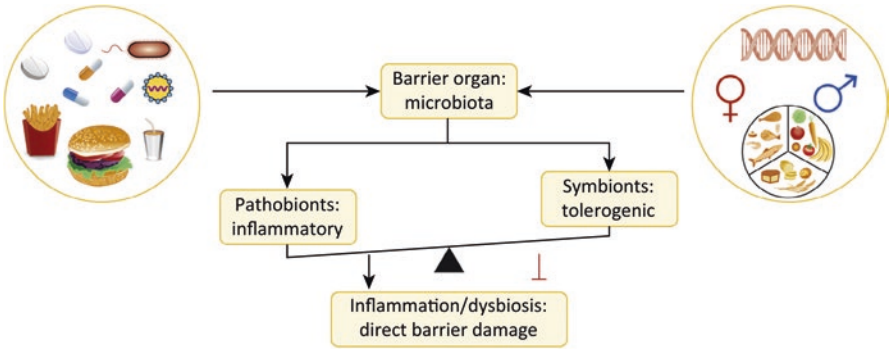

Fig. 3.1 Multiple factors influence the composition and function of the microbiota at barrier sites. The gut microbiota is profoundly affected by diet, medications, infectious agents, genetic makeup of the host, and also hormonal factors. These environmental and genetic influences shape the balance between pathobionts and symbionts, so that chronic inflammation ensues under certain autoimmune-prone conditions that leads not only to barrier damage (as shown in this figure) but also to distant autoimmune pathology, e.g., autoimmune thrombosis in various organs as in APS (The figure was adapted from Ref. [67])

arthropathy and colitis when HLA-B27 transgenic animals are kept germ-free [75]; in contrast, non-obese diabetic mice (prone to type 1 diabetes) are increasingly susceptible to autoimmune destruction of the pancreatic islet cells with increasing hygiene [76]; germ-free animals develop diabetes with an almost 100% incidence [77]. Environmental factors other than cleanliness, for instance, specific dietary components, can also impact the gut microbiota and therefore immune function and autoimmunity (Fig. 3.1) [67, 78–80]. Dietary effects on microbiota observed in other autoimmune diseases [81] may have a similar impact on APS.

Vieira et al. explored the gut microbiota in the (NZWxBXSB) F1 mouse, a spontaneous model of SLE and APS [81, 82]. Mice treated with an oral broad-spectrum antibiotic cocktail (vancomycin, metronidazole, neomycin, and ampicillin), or with vancomycin or ampicillin alone, did not develop serum aβ_2GPI IgG or die from thromboemboli [82]. Bacterial load (16S ribosomal DNA) monitored in the feces of the mice was profoundly suppressed by broad-spectrum antibiotic treatment. These data suggest that gut commensals may play a role in the (NZWxBXSB) F1 model of APS.

Cell Death

Apoptotic cells provide a potential natural target and immunogen for aPL as well as for most autoantibodies found in SLE [83–86]. The apoptotic cell surface contains anionic phospholipid (phosphatidylserine) not present on the surface of viable cells [87–89], enabling the interaction of phospholipid-binding proteins

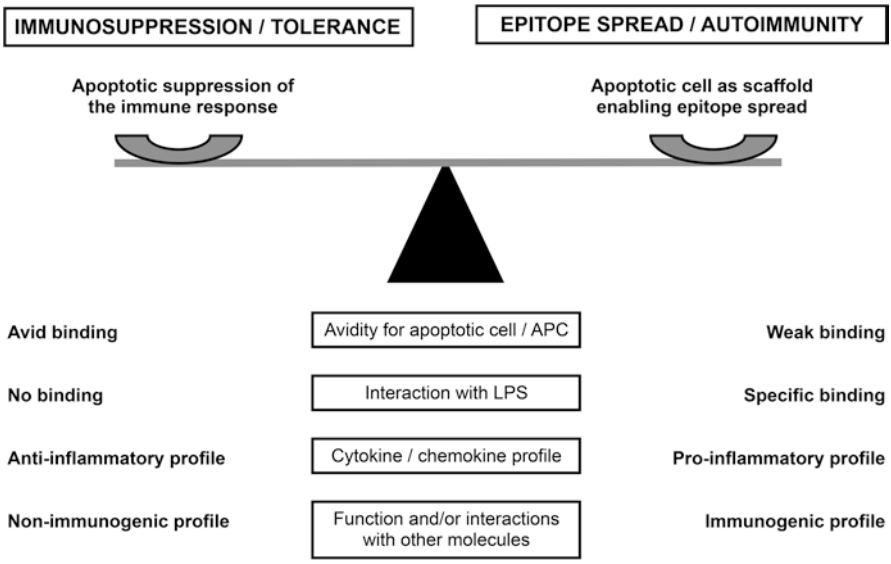

Fig. 3.2 Interaction of phospholipid-binding proteins with apoptotic cells: a balance between immunosuppression and autoimmunity. The hypothetical model proposes that the characteristics of a phospholipid-binding protein, and the nature of its interaction with apoptotic cells, can affect the balance between *immunosuppression/tolerance* and *epitope spread/autoimmunity*. On the one hand, apoptotic cells can suppress the adaptive response to an antigen when it is physically associated with the cells. On the other hand, apoptotic cells can serve as a scaffold that links surface-bound phospholipid-binding protein (e.g., β_2GPI) to other molecules (particularly lupus-associated autoantigens) on the apoptotic cell. Multiple factors determine whether the balance is tipped toward immunosuppression/tolerance or toward epitope spread/autoimmunity. This figure shows several potential factors (affinity/avidity for apoptotic cell/APC, interaction with LPS, cytokine/chemokine profile, and function of the phospholipid-binding protein and/or its interactions with other molecules) (*boxed text*, *center*). Characteristics of the phospholipid-binding protein that tip the balance toward immunosuppressive are listed on the *left side* of the figure, while those promoting epitope spread and autoimmunity are listed on the *right side*. *SLE*, systemic lupus erythematosus; *β_2GPI*, β_2-glycoprotein I; *LPS*, lipopolysaccharide; *APC*, antigen-presenting cells (This figure is reproduced with permission from Levine et al. (copyright 2014. SAGE Publications) [151])

such as β_2GPI [90] and prothrombin [91], both important target antigens of aPL (Fig. 3.2). Interaction of β_2GPI with apoptotic cells generates epitopes that are immunogenic in normal mice [92]. More recent findings indicate that β_2GPI is highly immunogenic when presented in the context of innate immune activation, such as that induced by bacterial LPS [93]. In fact, mice immunized with β_2GPI not only develop high levels of aβ_2GPI and aCL but also multiple SLE-related autoantibodies and lupus-like glomerulonephritis [93]. Salem et al. [94] showed that epitope spread in the autoantibody response from β_2GPI to multiple autoantigens is associated with a strong T cell response to β_2GPI, independent of the particular β_2GPI T cell epitope specificity. The authors propose that B cells specific for apoptotic cell-associated surface autoantigens take up apoptotic cells via antigen-specific surface IgG, leading to surface MHC class II-associated presentation of

multiple apoptotic cell-derived peptides, including those from β_2GPI. In this way, a β_2GPI-specific T cell can provide help to a B cell that internalizes an apoptotic cell with surface-bound β_2GPI [93, 94].

Recently, a form of cell death called NETosis has been implicated in several autoimmune diseases, including APS and SLE [95]. Neutrophil extracellular traps (NETs) are responsible for a form of cell death that is distinct from apoptosis or necrosis. Yalavarthi et al. [96] reported that freshly isolated neutrophils from patients with primary APS have higher levels of spontaneous NET release than do those from healthy control subjects. Furthermore, exposure of neutrophils from healthy controls to APS patient serum or IgG, or monoclonal aβ_2GPI IgG, stimulates NETosis. Neutrophil extracellular traps may be immunogenic, as they present captured microorganisms and autoantigens in an inflammatory milieu that stimulates an immune response [97]. However, the role of NETosis in aPL production remains unclear.

The Immunological Response

Antigenic Factors

Relatively little has been uncovered regarding the ontogeny of pathogenic aPL, but it is clear that both genetic and environmental factors play a role in its production. The role of genetics in APS will be addressed in Chap. 4; HLA and non-HLA genes likely confer a baseline risk for aPL generation and APS, while various environmental factors may augment and intensify this risk [98].

Animal models can elucidate the nature of the inducing antigen(s) and other factors involved in aPL production. Although early experiments focused on immunization of animals with putative antigens, such as cardiolipin, phospholipid alone failed to induce high-titer pathogenic aPL [99]. The discovery that β_2GPI, not phospholipid, was the main antigenic target of autoimmune aPL [100, 101] led to immunization with human β_2GPI, combined with cardiolipin or adjuvant, and the successful induction of aPL [99, 102]. In some cases, these antibodies had pathogenic effects [99]. More recently, de Laat et al. [103] showed that murine or human β_2GPI combined with cardiolipin, or misfolded β_2GPI itself, can trigger antibody to β_2GPI. Recombinant Domain I, but not Domains II–V, induced aβ_2GPI. Together these findings suggest that β_2GPI can be immunogenic when presented in a context in which immunogenic cryptic epitopes are exposed.

Another change in β_2GPI that may expose cryptic epitopes is increased oxidative stress, which can occur with infection and other pathologies [104]. Ioannou et al. [105] showed that the proportion of protective free thiol β_2GPI (which constitutes the majority of circulating β_2GPI) is low in APS patients compared to other autoimmune patients and healthy controls. The higher proportion of oxidized β_2GPI may result in limited ability to protect cells from oxidative stress [105] and increased T cell immunogenicity [106]. Thus oxidative stress might give rise not only to a more immunogenic β_2GPI but also a procoagulant microenvironment.

Innate Immune Factors

While the antigenic stimulus in aPL induction is important, the immune environment in which antigenic exposure occurs may be equally or more critical. Infections potentially provide both necessary ingredients: antigen, and an innate immune stimulus. In addition to providing antigenic peptides that mimic β_2GPI, infectious pathogens expose the host to TLR agonists like LPS, cytokine/chemokine release, and selective activation or destruction of lymphocytes [107–110]. Hence, an infectious organism may be a molecular mimic or, just as importantly, modulate the innate immune system.

Rauch and Levine [111, 112] suggest a hypothesis that highlights the central role of innate immune receptors, especially TLR4, in breaking tolerance. Mice immunized with β_2GPI and LPS develop high levels of aβ_2GPI, aCL, and multiple SLE-related autoantibodies, as well as glomerulonephritis [93, 94]. Pierangeli and coworkers [113] also showed that activation of TLR, in this case TLR7 and TLR9, induces aPL, as well as tissue factor production and thrombus formation, in autoimmune-prone PL/J mice treated with cytomegalovirus-derived peptides. All outcomes were highest in mice treated with both agonists. In SLE-prone $MRL^{lpr/lpr}$ mice, aPL titers were decreased in mice deficient in TLR7 alone, or both TLR7 and TLR9, but not TLR9 alone. These data support the hypothesis that innate immune receptors TLR4, TLR7, and possibly TLR9 are involved in the loss of tolerance to β_2GPI.

Regulatory Immune Factors

The break-in tolerance among APS patients may involve Treg dysfunction [114]. Peripheral blood mononuclear cells from healthy donors treated with increasing concentrations of aPL showed changes in T cell subsets, compared to cells treated with control IgG [114]. T helper2 (Th2) and Th17 cell frequencies were increased, while Th1 and Treg cell frequencies were decreased. A subsequent study in primary APS patients reported a reduced frequency of $CD4^+CD25^+foxp3^+$ Treg cells compared to controls [115]. These studies suggest that Th1/Th2 imbalance coupled with Th17 upregulation may play a role in aPL production and APS.

What Is Controversial and/or Unknown?

Natural Autoantibodies

Increasing evidence suggests that aPL, including aβ_2GPI, belong to the natural antibody repertoire [116–119]. Natural antibodies are antibodies present in the circulation without prior infection, vaccination, other foreign antigen exposure, or passive immunization. Natural antibodies are often directed against highly conserved epitopes or structures present in many species, such as clusters of charged molecules. They typically bind with low affinity to ligands of varying chemical composition and can react with a number of unrelated antigens (including self-antigens). It is

possible that natural antibody production is driven by bacteria living in the intestine [64]. Support for the identification of aPL as natural antibodies comes from the observation that normal healthy individuals without APS can have memory B cells that produce aPL [120].

As part of the innate immune system, natural antibodies can influence metabolic processes. In a series of elegant studies in mice, Fleming et al. showed that natural aβ_2GPI are involved in complement-mediated mesenteric ischemia/reperfusion-induced injury [121, 122]. Additionally, natural aPL may be involved in acute graft rejection after renal transplantation [123]. Natural antibodies play a role in the clearance of apoptotic bodies; β_2GPI and aβ_2GPI may be essential for the clearance of these cell remnants [124]. Recent epidemiological studies in a large cohort of SLE patients have shown that aβ_2GPI IgM protects against lupus nephritis [11]. In a recent study, de Mast and coworkers [12] extended these findings and showed that aβ_2GPI IgM protects against stroke. Up to 5% of the healthy population has benign, low-affinity aPL in their circulation, a prevalence that increases with age [125]. These observations suggest that low-titer and low-affinity natural aβ_2GPI present in healthy individuals may serve a protective role.

The natural antibody repertoire comprises two major subsets: an overt antibody population and a cryptic or latent population [126], the latter defined by unmasking in vitro with high salt solution, low pH, or oxidative agents. Thus sera negative for aPL can become positive after oxidation or heating to 56 °C [127, 128]. Apparently, slight modulations within the antibody binding site can change epitope recognition. As these studies have been done using serum or plasma, the role of serum/plasma factors cannot be excluded. The epitope recognized by pathogenic autoantibodies against β_2GPI is also cryptic and is present in Domain I of β_2GPI. Notably, this epitope has been completely conserved in mammalian evolution [25]. The factors promoting the transition of natural aβ_2GPI from benign to pathogenic remain elusive.

Infection

Helicobacter pylori (*H. pylori*), one of the most common bacterial human pathogens, colonizes gastric mucosa where it induces chronic inflammation of variable severity: superficial gastritis, peptic ulcer, gastric cancer, and mucosal-associated lymphoma [129]. Some intriguing data link *H. pylori* and aPL. Anti-β_2GPI screening of 50 patients with *H. pylori* infection revealed a prevalence of 33.3% positivity [130]. In another study, APS disappeared after *H. pylori* eradication [131], and in a third study, *H. pylori* infection appeared to affect fetal intrauterine growth [132].

Rheumatic fever (RF) and subsequent rheumatic heart disease represent a relatively common connective tissue disease caused by *Streptococcus pyogenes*. Molecular mimicry mainly between the pathogenic M protein and self-antigens is thought to be a mechanism for developing acute RF after streptococcal pharyngitis [133]. Rheumatic fever and APS share common clinical manifestations, such as carditis and chorea. The pathological spectrum of valve lesions found in RF and APS patients is similar, including non-infective verrucous vegetations (Libman-

Sacks endocarditis), thickening of valve cusps, and, occasionally, significant valve dysfunction [134]. Sydenham's chorea is another major feature of RF [135]. Chorea has been associated with aPL and APS in many case reports and small patient series [136]. Blank et al. [137] hypothesized that common clinical features between RF and APS may be the consequence of a cross-reactive epitope between the M protein and β_2GPI. Indeed, β_2GPI-related peptides TLRVYK and LKTPRV share homology with *Streptococcus pyogenes* M protein. β_2-glycoprotein I-related peptide TLRVYK inhibited the binding of anti-M protein antibodies from RF patients to M protein by 37%, while M protein inhibited the binding of aβ_2GPI to β_2GPI by 23% [137]. Furthermore, affinity purified aβ_2GPI from two APS patients with chorea bound to N-acetyl-b-D-glucosamine (GLcNAc), a streptococcal antigen targeted in RF, while β_2GPI inhibited anti-GLcNAc binding of IgG from these patients. The authors suggest that the considerable overlap of the antibody response in RF and APS patients supports the hypothesis that common pathogenic mechanisms underlie the development of cardiac and central nervous system abnormalities in both diseases.

The Microbiome

It remains to be shown that commensal microbes drive aPL production via cross-reactivity with β_2GPI. It is possible that several cross-reactive epitopes within the gut microbiome act in concert and that more than a single commensal mediates aβ_2GPI responses. Furthermore, cross-reactivity with gut commensals may not apply to all APS patients. It is also plausible that the microbiota provides factors, such as phospholipids, that lead to a conformational change in β_2GPI. Toll-like and other pattern-recognition receptors could also be triggered chronically by a steady supply of ligands from the microbiota. These mechanisms may require a leaky gut barrier, which appears to be present in some APS patients [138]. Finally, the role of the microbiota at other barrier sites, such as the skin or lung, is unexplored but could theoretically also trigger aPL production. Infection with pathogens at these sites might further contribute to dysbiosis (an imbalance in the microbiota) by disrupting the mucosal barrier and inducing commensal-specific memory T cells [139]. Overall, there are multiple mechanisms by which the microbiota may elicit or perpetuate the production of aPL.

Current and Planned Research

The role of the microbiome in human APS is still unexplored, but findings in the murine APS model [64, 82] suggest that gut commensals may be involved. Kriegel and coworkers, using a systematic homology search in vitro, identified a common human colonic gut commensal, *R. intestinalis*, as a potential candidate for mediating both T and B cell cross-reactivity in humans [140]. Protein extracts from *R. intestinalis* elicit marked proliferative responses in peripheral blood lymphocytes

from APS patients, particularly those who are HLA-DR53 positive. Clones of human β_2GPI-specific, gut-homing CD4 memory Th17 cells recognize *R. intestinalis* mimic peptides. This research will provide insight into whether a prevalent human gut commensal can sustain persistent T and B cell reactivity in APS patients.

Kriegel and coworkers are completing a longitudinal microbiome study of APS and control patients that includes profiling of all fecal commensal organisms, as well as analysis of which microbiota are coated with IgA, a marker for inflammatory commensals [141]. Commensal candidates that emerge from the human profiling studies will be transferred into germ-free mice to test whether aβ_2GPI can be elicited locally (in the gut) or systemically (in association with APS manifestations). Finally, germ-free rederivation of the (NZWxBXSB) F1 murine model of APS, with subsequent colonization with human microbiota from APS patients, would enable the evaluation of causal links with specific human commensals in a murine model of APS.

Future Research Directions and Group Conclusions

Infection

The Association Between Infections and aPL

The association of aPL with *H. pylori* and *Streptococcus pyogenes* needs further investigation, as does the homology of the pathogen-derived proteins with β_2GPI-related peptides.

Leprosy and Syphilis

Future research on aPL in leprosy should include detailed information on leprosy and leprosy immune reactions. The stage of anti-leprosy therapy, type of immune reaction, and concurrent immunomodulatory agents should be included in serology and aPL activity studies. Antiphospholipid antibody levels in multibacillary leprosy in relation to anti-leprosy therapy, reversal reaction diagnosis and treatment, and ENL diagnosis and treatment may give insight as to whether aPL are related to the pathogenesis of leprosy immune reactions.

The Microbiome

The microbiota represents a potential antigenic and stimulatory source of autoreactivity and could play a fundamental role in APS pathogenesis (Fig. 3.3) [64, 67]. Future studies should aim to confirm the presence and load of both pathological and

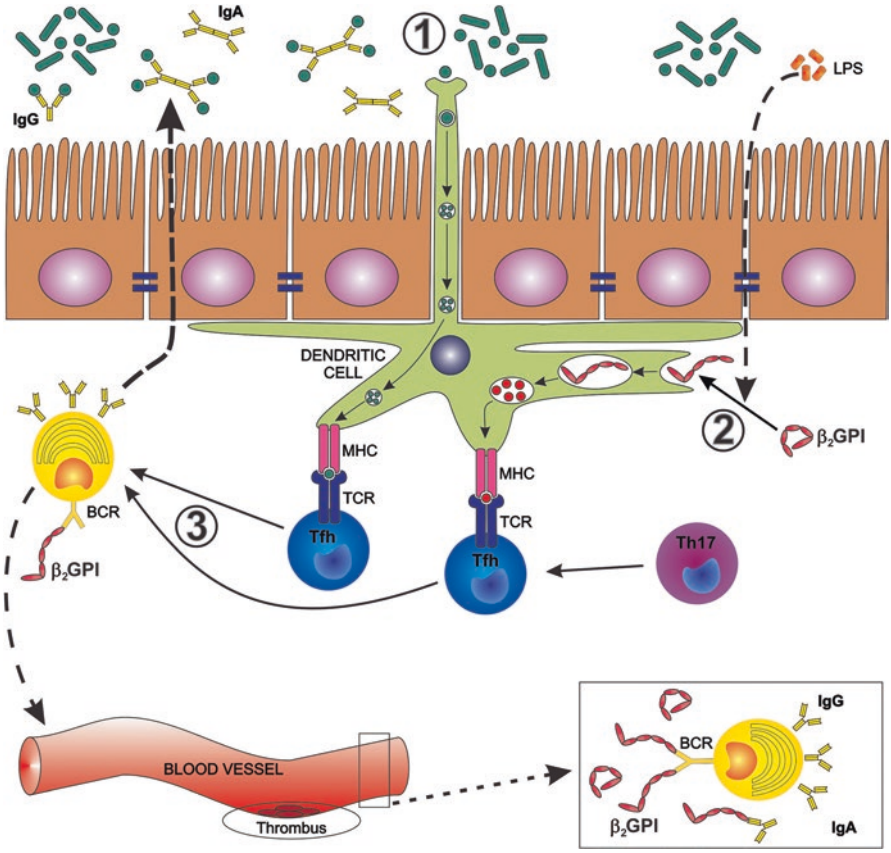

Fig. 3.3 Hypothetical model of how the gut microbiota might influence the pathogenesis of APS. Shown is the gut epithelial lining that excludes the microbiota (*green*) from the host. *Step 1:* Live commensals are taken up by antigen-presenting cells (e.g., a dendritic cell shown in *light green*) and presented to CD4 T cells in the local lymph node. *Step 2:* Lipopolysaccharide and phospholipids derived from the microbiota can also reach the host side and affect the conformational state of β_2GPI (*orange*) that is also processed and presented by antigen-presenting cells. *Step 3:* T helper subsets, in particular Th17 cells that are known to convert to Tfh in the gut, may recognize both β_2GPI and cross-reactive commensal antigens and subsequently assist autoreactive B cells to produce aβ_2GPI. These could be of IgA and IgG isotypes, thus feeding back not only into the gut by active IgA transport across the barrier but also diffusing into the systemic circulation where they initiate thrombus formation at sites injured or primed by a "second hit." (The figure is reproduced from Ref. [64]. The final publication is available at Springer via http://dx.doi.org/10.1007/s11926-014-0472-1)

beneficial microbiota that may mitigate disease. Future diagnostic and therapeutic applications that could develop from these studies range from novel biomarkers of disease risk to antibiotic, dietary, probiotic therapy, and commensal-depleting vaccines.

Cell Death

Future studies should elucidate the role of apoptosis and NETosis in the induction of aPL and APS. For instance, the mechanism by which apoptotic cells induce aPL remains unknown. For NETosis, it remains to be seen whether NETs are capable of inducing aPL and clinical features of APS.

The Immunological Response

Antigenic Factors Further investigation into the protective role of free thiol versus oxidized β_2GPI in APS patients may be important both for understanding pathogenic mechanisms and the development of therapeutic agents. The role of natural antibodies in APS also merits further investigation, both as potentially protective antibodies and as a source of pathogenic aPL.

Innate Immune Factors The importance of innate and regulatory immune factors in breaking immune tolerance and subsequent aPL production needs more in-depth investigation. Complementary approaches using murine models of APS and human biospecimens are important to ensure that findings are relevant to human disease.

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Chapter 4
Recent Advances in Understanding of the Genetics of Antiphospholipid Syndrome

Niti Goel and Thomas L. Ortel

Introduction

Multiple studies describe familial occurrence of antiphospholipid antibodies (aPL), with or without clinical evidence of antiphospholipid syndrome (APS). In addition, several studies report genetic risk factors associated with thrombotic complications or recurrent pregnancy loss (RPL) in patients with aPL. Given the heterogeneity of the clinical manifestations associated with this syndrome, it is likely that different genes in addition to acquired risk factors will be involved. Identification and characterization of gene variants associated with the development of APS will potentially enable the development of more finely targeted therapies. This chapter will present the recent advances in our understanding of the inherited risk factors associated with aPL and with APS.

Background: What Is Known?

Following the initial report by Harvey in 1966 [1], multiple studies identified the presence of aPL in family members of patients with APS and of patients with other autoimmune disorders, particularly systemic lupus erythematosus (SLE); only a small subset of these studies have had genetic analyses performed. In addition, genetic analyses of nonrelated individuals with aPL or APS have been performed. Taken together these reports, along with nonclinical data, provide a convincing basis for a disease model in which an inherited predisposition to the development and clinical expression of aPL promotes risk for thrombosis or pregnancy-related morbidity. Such information might provide insights into APS pathogenesis and more appropriate treatments. Supporting data are presented below.

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Nonclinical Models of Antiphospholipid Syndrome

The spontaneous production of aPL with assorted manifestations of APS, although not universally, has been described in various murine models of SLE (Table 4.1). Additional models, using knockout or knock-in mutations as well as induction of APS via immunization with phospholipid, have added to our understanding of the pathogenesis of APS. A recent study examined an overlapping mechanism linking the pathogenesis of APS and SLE by observing that deletion of the autoantigen β_2-glycoprotein I (β_2GPI) accelerated the lupus autoimmune phenotype [4]. Another study demonstrated the importance of the complement component C6 on thrombogenesis in APS by evaluating the effects of human aPL on thrombosis in C6-deficient (C6[−/−]) mice compared to wild-type C6(+/+) mice [9]. Both abrogation of thrombus formation in mice and diminished tissue factor (TF) expression and activity were seen in the aPL-treated C6(−/−) mice compared to treated wild-type mice. The possible involvement of CD36, a scavenger receptor expressed on monocytes, platelets, and endothelial cells, which recognizes multiple ligands, including phosphatidylserine, and regulates atherogenesis and thrombosis, was also evaluated for its role in the pathophysiology of thrombosis in APS [10]. Antiphospholipid antibody-induced TF expression was significantly suppressed on peritoneal macrophages from CD36-null mice compared to wild type, supporting the potential importance of this receptor in contributing to potential thrombosis in APS.

Another study used an annexin A5 knockout mouse model (Anxa5-KO) to evaluate its role in pregnancy-related morbidity [11]. The litter size was significantly reduced by deficient maternal annexin A5 production, with evidence of

Table 4.1 Murine models described for spontaneous antiphospholipid syndrome or antiphospholipid antibody production

Mouse model	Comments	Features of APS	Production of aPL
MRL/Mp-lpr/lpr (MRL/lpr) [2]	–	Yes	Yes
MRL-lpr/lpr (MRL/+) [2]	–	Yes	Yes
NZB [3]	–	Yes	Yes
BXSB-Yaa [4]	Features are of decreased frequency and intensity than in W/B F1 male progeny	Yes, males	Yes, males
NZW (female) × BXSB (male) F1 (W/B F1) [5]	–	Yes, males	Yes, both genders
C57BL/6 J [6]	Augmented by estrogen supplementation	No	Yes
AKR/J [7]	Autoimmune diabetes model	No	Yes, transient
NOD [8]	Autoimmune diabetes model	No	Yes

C57BL/6 J C57 Black 6 J, *lpr* lymphoproliferation spontaneous mutation, *MRL* Murphy Roths Large, *NOD* nonobese diabetic, *NZB* New Zealand Black, *NZW* New Zealand White, *Yaa* Y-linked autoimmune accelerator gene

placental thrombi formation and fetal growth restriction; these findings were ameliorated with treatment with heparin. These results support the hypothesis that the maternal supply of annexin A5 to the circulation is necessary for maintaining a fully intact pregnancy. An additional study examined the association of prothrombotic factor V Leiden (FVL) on APS manifestations [12]. Evaluating a mouse model of central nervous system manifestations of APS with a knock-in transgene for FVL, an increase in aPL levels and a number of behavioral/cognitive dysfunction and neurodegenerative changes associated with these autoantibodies were noted in the FVL APS mice. These effects were linked to gene dosage, and were thus significantly more pronounced in homozygous than in heterozygous mice, supporting the synergistic impact of other thrombotic risk factors in the manifestations of APS.

Antiphospholipid Syndrome Family and Population Studies

Clinical Phenotype Evaluations

Family and population studies may describe clinical phenotypes, including laboratory values, or may delve further into genotypes, the latter more systematically assessing inherited risk for APS. In exploring clinical phenotypes, family history identifies individuals with clinical manifestations associated with the syndrome [13]. An inherent limitation to this method is that by collecting retrospective clinical data without prospective confirmation, one may miss other etiologies that explain the clinical manifestations, such as a separate inherited prothrombotic risk factor (e.g., FVL) in a family member with venous thromboembolism. Several families have been reported with aPL combined with a second hematologic defect, like FVL [14], factor XII deficiency [15], and a factor IX inhibitor [16], with additional data supporting a risk synergy between classic inherited thrombophilias and APS.

Multiple families have been described in which two or more members have APS. Most of these family investigations are small, however, with details obtained for the affected family members and relatively limited information available concerning unaffected individuals [17–24]. Families with members who have aPL and some of the less obvious clinical manifestations associated with these autoantibodies, such as thrombocytopenia and/or cardiac valve disease, have been described [14, 25].

Another clinical phenotype approach to search for inheritance patterns is to determine the frequency of elevated aPL levels in family members of patients with APS or autoimmune disease [20, 25–28]. Such studies suggest a familial propensity but underscore difficulties in validation of clinical data and likely underdiagnose APS and underestimate its prevalence both in proband families and in control populations [29]. Because spouses of SLE patients may have abnormal LA tests, such studies also demonstrate that environmental factors may play a role in the development of aPL [30].

Sneddon syndrome is livedo reticularis and cerebrovascular ischemic lesions, frequently in association with aPL [31]. Multiplex families with the syndrome and aPL have been described [32–34], including a large family with several individuals with strokes at an early age [35]. In contrast, in at least one family with familial Sneddon syndrome, the clinically affected individuals did not have aPL, suggesting that Sneddon syndrome, at least in some cases, is a separate clinical entity from APS [36].

Genotype Evaluations

While genetic analyses related to nonfamilial APS are frequent, few exist that evaluate families. Goel and colleagues reported the first large-scale genetic study of multiplex APS families [37]. Their segregation analysis of seven families, with 30 of 101 family members with APS, suggested either a dominant or codominant model for disease inheritance. It failed, however, to find linkage to human leukocyte antigen (HLA) and other candidate genes including β_2GPI, antithrombin, factor V, and Fas. A limitation of this study is that the clinical diagnostic criteria for the syndrome were based on a semiquantitative scoring index that differed from the subsequently developed [38] and revised International APS Classification Criteria [39], and likely identified certain individuals as having APS who would not meet current diagnostic criteria.

HLA Associations

A hallmark of autoimmune conditions is the strong association of many of these diseases with genes in the major histocompatibility complex (MHC) region. Many HLA antigens have been associated with aPL, primary APS (PAPS), and APS associated with other autoimmune diseases (Tables 4.2 and 4.3), producing a complicated and confusing dataset for APS. Many of these reports are small case series, occasionally even single multiplex families, and testing for aPL is frequently incompletely documented (e.g., single test performed, positive cutoff values not provided, and positive results not repeated to confirm). Confirmation of meeting classification criteria for APS is also limited as many of the studies were performed before the existence of criteria. Some of these studies also lack, or fail to specify, appropriate ethnic- or gender-matched controls for the populations studied, where gender or ethnicity might account in part for the frequency of associations.

Non-HLA Associations

β_2-glycoprotein I is a phospholipid-binding protein that has been identified as a major antigen in patients with APS. The protein sequence for this plasma protein was published in 1984 [40], and the complementary DNA sequence in 1991 [41, 42]. Several polymorphisms have been identified in the protein [43], including three in the phospholipid-binding fifth domain (Val/Leu^{247}, Cys/Gly^{306}, and Trp/Ser^{316}). Studies have investigated the relationship between polymorphisms in β_2GPI and

Table 4.2 Positive associations between human antiphospholipid syndrome populations and human leukocyte antigen alleles

Model	Statistically significant findings based on multivariate analyses[a]
SLE population studies	aCL: DRB1*0402 (DR4), DR7 SAPS: DR7 Worsened survival: DQw7
SS population study	aCL: DR2, DR3 (Note in other settings, DR2 has been negatively associated with aβ$_2$GPI production in APS)
APS (both primary and secondary) population studies	LA: DQB1*06 (DQ6), DQB1*0301 (DQw7) aCL: DRB1*0402 (DR4), DR7 Anti-β$_2$GPI: DQA1*03, DQB1*0302 (DQ8), DQA1*0401, DQB1*0604/5 (DQ6, in African Americans), DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 haplotype APS: DR7, DMA*0102
PAPS population studies	PAPS: DR5 (DRB1*1201), DRw53, DQ7 PAPS and aβ$_2$GPI: DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 haplotype

aCL anticardiolipin antibody, a*β$_2$GPI* anti-β$_2$-glycoprotein I antibody, *APS* antiphospholipid syndrome, *LA* lupus anticoagulant, *PAPS* primary antiphospholipid syndrome, *SAPS* secondary antiphospholipid syndrome, *SLE* systemic lupus erythematosus, *SS* Sjogren's syndrome

[a]Clinical conditions and parameters include the presence of a specific aPL in the absence of any clinical manifestations of the syndrome as well as the presence of the full clinical syndrome (i.e., APS)

anti-β$_2$-glycoprotein-I (aβ$_2$GPI) in various ethnic groups. Gushiken and colleagues found no association between aPL (LA or aβ$_2$GPI) and the Cys/Gly^{306} and Trp/Ser^{316} polymorphisms which disrupt the ability of the protein to bind to anionic phospholipids [44], in patients with SLE and/or APS [45]. Similarly, Camilleri and colleagues found no relationship between the Trp/Ser^{316} polymorphism and aPL in patients with thrombosis [46]. Palomo and colleagues did find a significant relationship between this polymorphism and venous and arterial thromboses, but not with aPL, in Chilean patients [47]. Similar findings were reported by Pardos-Gea et al.; i.e., polymorphisms in Trp/Ser^{316}, by means of statistically significant associations for an increased S allele and T/S genotype in Spanish Caucasian patients, might play a role in the pathogenic development of PAPS, but not via increased production of aβ$_2$GPI or other aPL [48].

The Val/Leu^{247} polymorphism has been more extensively studied. This variant causes a conformational change in β$_2$GPI [49] hypothesized to result in the exposure of cryptic epitopes [50], theoretically providing a likely target for autoantibodies. Initial studies identified a relationship between this polymorphism and aβ$_2$GPI in patients with APS, although one study identified this association in Caucasian patients with primary but not secondary APS [51], and a second study identified the association in Asian patients with APS but not Caucasian or African American patients [52]. To evaluate not only potential differences related to ethnicity or the presence of primary versus secondary APS, a recent meta-analysis by Chamorro et al. of eight previous studies, comprising 488 patients meeting classification criteria for APS and 923 controls from eight countries, evaluated the association of APS, PAPS, aβ2GPI, and/or thrombosis with the Val/Leu^{247} polymorphism [53],

Table 4.3 Human leukocyte antigen associations reported for nonfamilial and familial antiphospholipid syndrome

Author	Ethnicity/ location	Patients (*n*)	Control (*n*)	Phenotype associations evaluated	Results
Nonfamilial					
Panzer et al. [97]	Austrian	27 mixed (PAPS, 22; SAPS due to SLE, 4; SLE only, 1)	637	aCL, LA, antiplatelet antibodies	Increased frequency of *HLA-DQB1*06* with LA after correction for multiplicity testing
Freitas et al. [98]	Brazilian	123 mixed (PAPS, 34; SAPS due to SLE, 35; SLE only, 54)	166	APS (by criteria) and SLE	PAPS with nonsignificant increased frequency of DR53-associated alleles; association of SAPS with HLA-DRB1*03 was due to the association with SLE and not aCL and suggested that the HLA class II profile of PAPS is different from that of SAPS
McHugh et al. [99]	British	46 SLE	318	aCL	DR4 in 7/8 aCL-positive SLE patients; associations of NS after multiplicity testing
Asherson et al. [100]	British	13 PAPS	69	aCL, LA, PAPS	DRw53 positively associated with PAPS and aCL; DR4 positively associated with PAPS; DR3 not present in any patients vs controls; associations NS after multiplicity testing
Sanchez et al. [101]	British Caucasian	133 mixed (PAPS:51; SAPS due to SLE:42; SLE only:40)	109	any aPL, aCL, aβ_2GPI, aPT, LA	The distribution of DMA alleles was significantly different between all APS patients or SAPS patients and controls with the increase in **DMA*0102** showing the strongest contribution. The distribution of DMA alleles in PAPS or SLE only patients was not significantly different from that in controls
Bertolaccini et al. [102]	British Caucasian	82 mixed (all with aPL and 74 with APS: PAPS:53; SAPS due to SLE:29; SAPS due to other: 2)	177	aβ_2GPI, LA, aPS/PT	HLA-DQB1*0301/4 associated with aPS/PT with or without aβ_2GPI with increased frequency. Uncertain if multiplicity corrections applied
Caliz et al. [103]	British Caucasian	83 mixed (PAPS:53; SAPS due to SLE:30)	177	aβ_2GPI, APS features	**DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302** and DQB1*0303-DQA1*0201-DRB1*0701 haplotypes showed significantly positive correlations with APS that were NS after correction. The association of the former was significant after correction only in PAPS with aβ_2GPI.

Goldstein et al. [104]	Canadian	107 mixed (PAPS:16; SLE with aPL:19; SLE without aPL:72)		aCL, LA, APS	HLA-DR4 and the linked DR53 were significantly increased in PAPS compared to SLE. In patients with aPL (SLE and PAPS) compared to patients with SLE without aPL, associations were found with HLA-DR53 and to a slightly lesser degree DQ7. The HLA-B8, DR17, DQ2 haplotype closely associated with SLE was significantly decreased in both SLE with aPL and with PAPS. No evidence of association with C4A deficiency alleles. No correction for multiplicity testing appears to have been applied
Hartung et al. [105]	Central Europe	314 SLE (SAPS:17)	–	aCL IgM and IgG, APS	aCL IgM were positively associated with **DR4**, DR7, **DRw53**; DR7 association NS after corrections for multiplicity testing; no evidence for significant associations with DQ or C4 alleles or for aCL IgG or APS.
Galeazzi et al. [106] and Sebastiani et al. [107]	European	577 SLE	Unknown	aCL, aβ_2GPI, APS features	aCL were positively associated with HLA-DRB1*04, -DRB1*07, -DQA1*0201, -DQA1*0301, -DQB1*0302, -DRB3*0301, -DPB1*1501, and -DPB1*2301. aβ_2GPI were positively associated with HLA-DQB1*0302, -DPB1*0301, and -DPB1*1901. HLA-DQA1*0501 and -DRB3*0202 showed a negative association with aCL. Although aCL and aβ_2GPI were associated with HLA-DRB1*0402 and -DRB1*0403, only the **-DRB1*0402** association was still positive with med-high titer aCL after correction for multiplicity testing. No associations were found with disease manifestations after correction
Ioannidis et al. [108]	Greek	67 mixed (PAPS:37; SAPS:30)	246	aβ_2GPI	aβ_2GPI response was positively associated with HLA-DQA1*03 (in particular *0301) and the HLA-DRB1*1302-DQB1*0604 haplotype, while protection against developing an aβ_2GPI response was related to the HLA-DRB1*0101-DQA1*0101 haplotype and the HLA-DRB1*1101 allele. Correction for multiplicity testing doesn't appear to have been applied

(continued)

Table 4.3 (continued)

Author	Ethnicity/ location	Patients (*n*)	Control (*n*)	Phenotype associations evaluated	Results
Savi M et al. [109]	Italian	109 mixed (PAPS:19; SLE with aCL:36; SLE without aCL:44)	2 groups: 319 and 633		aCL positively associated with DR7; DR2 increased among aCL(−);uncertain if multiplicity corrections applied
Sebastiani et al. [110]	Italian	44 SLE	100	IgG or IgM aCL	No significant associations with DR alleles found
Trabace et al. [111]	Italian	49 women randomly chosen from 120 with RPL (aCL[+]:25; aCL[-]:24)	100 including 54 men	aCL	HLA-DR7 in aCL(+) vs aCL(−) (NS after adjustment for multiple comparisons) but no differences vs healthy controls
Hashimoto et al. [112]	Japanese	145 SLE (29 with aβ_2GPI)	113	aβ_2GPI	The frequency of DRB1*0901 (DR9) was lower in SLE patients than in healthy subjects. SLE patients with aβ_2GPI-showed significant positive association with DRB1*0901 compared to those without aβ_2GPI; associations NS after correction for multiplicity testing
Vargas-Alarcon et al. [113]	Mexican	17 PAPS (all with aCL)	100	PAPS	Significant positive association of **HLA-DR5** with trend for DR5 subtype of -DRB1*1201. Increased frequency of DR52 also noted (NS). No associations seen with DQ7, DR53, DR7
Granados et al. [114]	Mexican	80 SLE	378 first-degree relatives; 50 married couples without SLE	aCL, APS	After multiplicity correction, positive association with aCL and APS noted with **DR7**
Camps et al. [115]	Southern Spain	19 PAPS	261	aCL and LA, APS features	**DQ7 and DRw53** associated with PAPS after multivariate analysis. DR4 numerically positively associated with high-titer IgG aCL, whereas DR7 was associated with low- or medium-titer IgG aCL. No HLA associations with LA. DQ1 significantly positively associated with migraine; DQ6 with RPL; DR1 with HTN and epilepsy (multiplicity corrections do not appear to have been applied to APS feature analyses)

Wilson et al. [116]	US: AA	44 SLE	38	aCL	A statistically significant association with IgG aCL and C4-null allele was seen. No correction for multiplicity testing was performed
Arnett et al. [117]	US	20 with LA (PAPS, 8; SLE, 9; SS, 2; SSc, 1)	139	LA	**HLA-DQw7 (DQB1*0301)** linked to HLA-DR4 or DR5 significantly associated after correction in all LA(+) patients and SLE LA(+) patients; among the HLA-DQB1 *0301 (DQw7)-negative patients, all possessed HLA-DQw8 (DQB1 * 0302) and/or HLA-DQw6 (DQB1 *0602 or DQB1 *0603) alleles
Asherson et al. [118]	US	65 SS (5 males)	150 white women	aCL (including IgA)	Significant positive association after correction between aCL and **HLA-DR2/DR3**—these were also associated with anti-Ro/SSA. No increased occurrence of haplotype DR2 or DR3 was noted for the SS patients vs controls, suggesting that gene interaction between DR2 and DR3 may play a part in the production of aPL in SS patients. Of the 13 subjects with aCL, 11 had IgA, 1 IgM, and 3 with IgG aCL. None had high titer IgM or IgG aCL. No subject had LA
Arnett et al. [119]	US: 3 ethnic groups (white, AA Mexican-American)	262 mixed (PAPS, 48; SAPS due to SLE, 70; SLE only, 126; other SAPS, 4; other CTD, 14)	393	aβ_2GPI	After correction for multiplicity, in whole patient group: **DR2, DRB1*1501 and/or 1503 (DR2), and DQB1*0602 (DQ6)** negatively associated with aβ_2GPI; **DQA1*03, DQB1*0302, and DQA1*0401** positively associated with aB2GPI. In assessing aβ_2GPI vs controls: **DQA1*0101 (DR1)** negatively associated and **DQB1*03 and DQB1*0302** (DQ8-linked to DR4) positively associated with aβ_2GPI. In AA patients, **DQB1*0604 or 0605** positively associated and **DQB1*0602** negatively associated with aβ_2GPI. In Mexican Americans, **DQA1*03** positively associated with aβ_2GPI

(continued)

Table 4.3 (continued)

Author	Ethnicity/ location	Patients (*n*)	Control (*n*)	Phenotype associations evaluated	Results
Galeazzi et al. [120]	Unknown	42 SLE	107	aCL	DPB1*0301 and/or DPB1*1401 after correction were statistically significantly increased in aCL-positive but also Sm/RNP-positive patients compared with 107 healthy controls; this was also statistically significant in aCL-positive versus aCL-negative patients, but not maintained after correction
Gulko et al. [121]	US, mixed ethnicities and races	139 SLE	None	aCL, APS features, survival	**HLA-DQw7** and thromboembolic events statistically significantly and independently in multivariate analyses adversely affected survival. No HLA association of DR or DQ alleles with aCL found
Familial					
Bhattacharya et al. [122]	British	8 members of a single family, 2 with APS, one with lupus nephritis	–	LA	The proband and father had APS; a paternal aunt had lupus nephritis. Five members displayed LA; none had aCL except the proband. Six members shared the same haplotype, A30, B13, DRB1–07 (DR7), and DQB1–02 (DQ2): four of these had LA.
Dagenais et al. [123]	Canadian, English	14 members of a single family, some with AID and/ or aPL; proband had APS	–	aCL, LA	The proband and 4 other family members shared the HLA-B60, DR4 haplotype; of these the proband had APS and 3 others were aCL positive without symptoms. No one without the haplotype had aCL
Hudson et al. [124]	Canadian, Native American	8 members of a single family, 4 with aCL	–	aCL	3 of 4 with aCL had DRB1*14, but 1 nonaffected family member did also

Bridey et al. [125]	French	13 members of a single family, 2 including proband with PAPS	–	aCL, LA, aβ_2GPI	Haplotype A11B51 C4A3BQ0 DR4 DRw53 DRB1*0402 was shared by proband and affected brother and seven other family members. Proband and brother were documented to have LA and aCL; proband also had aβ_2GPI. Two additional asymptomatic members with the haplotype had aβ_2GPI. Three of the 4 asymptomatic family members with borderline LA results shared the haplotype
Lousa et al. [126]	Spanish Caucasian	19 members of a single family, 13 HLA-typed; 2 with PAPS including proband who also had Sneddon syndrome, 1 with livedo reticularis; 10 had LA and/or IgM aCL	–	aCL, LA	The proband and father (as well as 1 asymptomatic nephew negative for both aCL and LA) had an HLA-A30-B13-Bw6 haplotype. In addition, an HLA-Bw6-DQ1 association was present in all the typed members of this kindred
May et al. [127]	US	8 members of a single family, 3 affected (mother and identical twins) with SAPS due to SLE	–	aCL, LA, APS features	aCL and LA present in all affected subjects with haplotype of DR4, DRw53, and DQw7, but haplotypes also present in a nonaffected brother and nonaffected sister; no evidence of C4A or C4B deficiency
Goel et al. [37]	US	101 members, 30 affected from 7 families	–	APS	No HLA associations found
Wilson et al. [128]	US Caucasians	38 members, 19 with AID (including PAPS but not SLE) or autoantibodies from three families	33	aCL	In ten members with aCL, all had 1 or 2 DQB1 risk alleles, and 9 of 10 had 1 or 2 C4 deficiency alleles

Items in bold text refer to findings which remained significant after correction for multiplicity testing and/or multivariate analyses

AA African American, *aCL* anticardiolipin antibody, *AID* autoimmune disease, *aPL* antiphospholipid antibodies, *aβ_2GPI* anti-β_2-glycoprotein I, *APS* antiphospholipid syndrome, *CTD* connective tissue disease, *HLA* human leukocyte antigen, *IgG* immunoglobulin G, *LA* IgM immunoglobulin M, lupus anticoagulant, *NS* not significant, *PAPS* primary antiphospholipid syndrome, *RPL* recurrent pregnancy loss, *SAPS* secondary antiphospholipid syndrome, *IgM* immunoglobulin M, *SLE* systemic lupus erythematosus, *SS* Sjogren's syndrome, *SSc* systemic sclerosis

determining that patients with APS had a significantly higher prevalence of the Val/Val genotype when compared with controls, with an enhanced association in those with aβ2GPI. No associations were found for the Val/Val phenotype and thrombosis, ethnicity (Caucasians with APS), or PAPS, but these analyses were limited by the small number of studies reporting these data. A separate meta-analysis evaluating the Val/Leu^{247} polymorphism included 10 studies with a total of 1507 patients and 1450 controls [54]. A limitation of this meta-analysis was that it did not confirm that patients met classification criteria for APS as done in the Chamorro meta-analysis. Only six studies were common to both meta-analyses. Despite the differences, the latter study confirmed the findings of the former except the latter also demonstrated an association of the polymorphism with thrombosis.

Several other genes have been studied in focused attempts to identify relationships with aPL and APS (Table 4.4). Fredi and colleagues studied 169 Italian patients with PAPS for polymorphisms in non-HLA genes that are associated with increased susceptibility for lupus, including *IRF5*, *STAT4*, and *BLK* [63] and found a strong genetic association with PAPS for *STAT4* and *BLK* and a weak association for *IRF5*. In a similar study, 48 single nucleotide polymorphisms (SNP) from 40 candidate loci for lupus were typed in a cohort of 208 Italian and Hungarian SLE patients and 152 controls [65]. No associations between secondary APS and *IRF5*, *STAT4*, or *BLK* were found; weak associations that did not persist after multivariate analysis were found with *NCF2* and *TYRP1*. In contrast, in separate studies, a *STAT4* polymorphism was associated with primary as well as secondary APS [58], and a different *STAT4* polymorphism was associated with aPL and ischemic cerebrovascular events in Swedish patients with lupus [59]. These conflicting data suggest that while genetic variants that increase the risk for the development of PAPS may overlap with those that increase the risk for the development of SLE, larger studies are

Table 4.4 Non-HLA genetic associations for human antiphospholipid syndrome populations from non-genome-wide studies

Model	Statistically significant findings[a]
SLE population studies	aPL: BF*F allotype (alternative complement pathway) protective against aPL development [55] aPL: *PDCD1* intron 4 polymorphism [56]
aPL-positive population studies	Thrombosis: *LDLR*, *PCSK9* [57]
APS (both primary and secondary) population studies	APS: CD36 deficiency less frequent [10] APS: *STAT4* [58] aPL: *STAT4* [59] RPL and aPL: *ANXA5* M2 haplotype [60] RPL but not aPL: *ANXA5* H3 haplotype [61] PAPS over SAPS: FcγRIIA-R/H131 HH homozygosity [62]
PAPS population studies	PAPS: *IRF5* [63] PAPS: *BLK* [63] PAPS: *STAT4* (different alleles) [58, 63]
aPL family study	Toll-like receptor 4 (TLR4) gene polymorphisms protective against thrombosis [64]

aPL antiphophospholipid antibody, *APS* antiphospholipid syndrome, *PAPS* primary antiphospholipid syndrome, *RPL* recurrent pregnancy loss, *SAPS* secondary antiphospholipid syndrome, *SLE* systemic lupus erythematosus

[a]Association is one of increased risk unless otherwise specified

needed to determine both those genes which truly contribute to heritable risk and why different disease phenotypes manifest.

Such studies appear to be underway—with both recent technologic advances and increasing worldwide collaborations to combine genetic repositories, genome-wide association studies (GWAS) are becoming more commonplace. Ramos and colleagues undertook a GWAS of 1506 individuals from 229 multiplex lupus pedigrees in an effort to map genes that contribute to the production of several autoantibodies encountered in patients with lupus, including aPL [66]. Several autoantibodies, including IgM aPL, exhibited a strong familial aggregation in these lupus pedigrees, but IgG aPL did not. A region highly suggestive for linkage to IgM aPL was identified on chromosome 13q14 particularly for European American, but not African American, pedigrees. Of the potential candidate genes in this chromosomal region, associations have been shown with atopic diseases [67], primary biliary cirrhosis [68], insulin-dependent diabetes mellitus [69], and Aicardi-Goutieres syndrome [70]. The last is potentially relevant as a rare neurologic disease which has been demonstrated to overlap with SLE [71] and APS [72], and is associated with overproduction of interferon alpha.

A similar but separate GWAS was performed by Kamboh et al. to identify candidate loci for the three main aPL, namely, anticardiolipin (aCL), lupus anticoagulant (LA), and aβ_2GPI in female SLE patients of European ancestry [73]. Analyses were performed for each aPL type individually (670 individuals with aCL, 708 with LA, and 496 with aβ_2GPI) as well as for the 100 subjects with two or more aPL types present versus 227 individuals who were negative for all three antibodies. For each aPL type, several highly suggestive non-HLA loci were identified including loci harboring *DYNLRB2* and *SESTD1* for individuals positive for at least two aPL; *PELO*, *SGIP1*, and *LCA5* for aCL; *MICAL3*, *FAM176A*, and *DSTN* for LA; and *MYO16*, *PDE1C*, *TANK*, *FLJ42392*, and *MACROD2* for aβ_2GPI. In contrast to the Ramos study, no linkage with chromosome 13q14 was determined for any aPL or combination of two aPL. Similar to the Ramos study, no linkage association reached the defined threshold of genome-wide significance, and no linkages to *IRF5*, *STAT4,* or *BLK* were reported. This study replicated previously reported HLA as well as β_2GPI (apolipoprotein H, APOH) polymorphism findings with similar magnitudes of association; as such these findings served as positive controls for the GWAS. The authors concluded however that the relative weakness of these associations in contrast to the identified non-HLA loci indicated that HLA genes and APOH were not among the top loci to explain genetic predisposition to aPL production.

While the aforementioned GWAS were performed in SLE cohorts, yet another aimed to identify genetic factors associated with aPL (IgG and IgM aCL, IgG and IgM aβ_2GPI, and aβ_2GPI domain 1 IgG) in 5000 patients from a German population cohort [74]. In contrast to Kamboh et al., this study determined significant associations which met the definition of genome-wide significance for aβ_2GPI IgG and APOH on chromosome 17. They also confirmed the genome-wide significance of an association between aβ_2GPI domain 1 IgG with *MACROD2* on chromosome 20, which had been a candidate gene suggested by Kamboh et al. Eleven other genes were shown to have suggestive associations in common with those determined by Kamboh et al., raising the possibility that regardless of APS being primary or secondary, the propensity to produce aPL may be driven by specific genes.

In an alternative approach to GWAS, genome-wide analyses can be undertaken evaluating copy number variants (CNV), genomic variants that may contribute to the genetic basis of human disease susceptibility. Array comparative genomic hybridization is a molecular cytogenetic technique for the detection of chromosomal copy number changes on a genome-wide and high-resolution scale. Using such a technique and then using published GWAS data to identify susceptibility genes, Ochoa and colleagues, in a Spanish Caucasian population, identified the 12q24.12 locus and, more specifically, within this region, a *TAC* (as opposed to *CGT*) risk haplotype comprising one SNP in the *SH2B3* gene and two SNPs in the *ATXN2* gene, as having the strongest associations with thrombotic APS [75].

Taken together, the genome-wide studies performed to date reveal previously unrecognized genes and their possible contributory role to the pathogenesis of APS. Such findings should stimulate further research into the role of *MACROD2* and other candidate genes in contributing to the APS phenotype.

Genetic Modifiers of Prothrombotic Risk

It may be difficult to distinguish between the impact of genes specific to autoimmune-mediated thrombosis and those with more direct impact on coagulation interactions in people who have both types of risk factors. This domino theory of inherited thrombophilic risk has been addressed in investigations that examine the impact of other known inherited prothrombotic states on thrombosis in patients who have aPL [76]. Factor V Leiden, the most frequently investigated, is associated with increased risk for thrombosis in multiple studies [77–79] and in a large meta-analysis [80]. Data supporting the prothrombin G20210A polymorphism and increased thrombotic risk in patients with aPL are less convincing [79]. Inherited deficiency states of the natural anticoagulant proteins (e.g., antithrombin, protein C, and protein S) are rare and have seldom been described in patients with APS (although acquired deficiency states have been). Multiple other prothrombotic risk factors have also been studied in patients with aPL and thrombosis, including TF pathway inhibitor [81], plasminogen activator inhibitor-1 [82], plasminogen activator inhibitor-2 [83], factor XIII-A subunit Val/Leu^{34} [84], and platelet surface glycoprotein receptors [85, 86]. These prothrombotic risk factors are not directly associated with aPL or APS but may modify thrombotic risk in a patient who has these autoantibodies.

These genetic studies provide a sound basis for the hypothesis that multiple interacting inherited factors may increase or decrease the net risk for an individual to develop APS. These factors may involve components of inflammation, tolerance mechanisms, or immune clearance (structural variants in autoantibodies and their receptors on myeloid cells). Such gene variants may overlap with risk factors for other autoimmune diseases such as SLE and may explain the high risk of secondary APS in SLE patients. Additional factors may be needed to promote specific aPL. Both environmental and genetic factors may play a role in the development and perpetuation of antibodies directed to target APS antigens. Finally, since aPL are associated most strongly with thrombotic risk, it makes sense that additive risks for thrombosis may be found when aPL are combined with known genetic thrombophilic variants.

What Is Controversial and/or Unknown?

Although laudable attempts have been made to provide consistency for studies of aPL by defining a manageable, sensitive, and reasonably specific set of consensus criteria for diagnosis, the full spectrum of antibodies which may be involved in autoimmune thrombosis is unknown and is likely to be much wider than the most clinically useful group included in these criteria (LA, aCL, and aβ_2GPI) [39]. Consideration of additional autoantibodies as part of the same syndrome may not increase the sensitivity of diagnosis by a great degree, but may be important in understanding the full genetic basis and pathogenesis of the disorder.

Both family and nonfamily studies have limitations, especially related to clinical phenotypes described before or during the evolution of the classification criteria for APS [38, 39]. These limitations include screening for only a single type of autoantibody (e.g., aCL or LA); determining aPL status on a single occasion; failure to evaluate for other prothrombotic conditions; and inclusion of subjects with "low-positive" results from enzyme-linked immunosorbent assays. Other issues identified with analyses are retrospective and possibly incomplete data collection from family members of the proband regarding clinical manifestations; lack of adequately described control populations; failure to differentiate results between primary and secondary APS subjects; evaluation of associations with aPL rather than with APS; failure to differentiate between different types of aPL in the results; focus on either pregnancy-related morbidity or vascular thrombosis but not both or combining these subjects with failure to subgroup results for subjects with primarily one or the other APS manifestation; and consideration of racial and or ethnic differences in gene prevalence and data generalizability.

Current Research and Future Directions

Gene Expression Profiling

A recent area of research has focused on gene expression profiling. Whereas various genetic polymorphisms may be associated with clinical phenotypes, the actual downstream regulation of genes may have a more direct impact on the latter. Understanding these downstream effects will undoubtedly aid our understanding of the pathogenesis of APS. Indeed, increasing evidence is being generated related to the expression of various pro-inflammatory, prothrombotic, and pro-atherosclerotic markers in APS [87], including, but not limited to, interleukin-6 [10, 87], TF [10, 87, 88], type I interferon [89], E-selectin [88], low-density lipoprotein receptor [57], and tumor necrosis factor α [74], as well as a gene with an unknown theorized pathogenetic mechanism related to APS, e.g., neuron navigator 3 [74].

MicroRNAs and Antiphospholipid Syndrome

MicroRNAs (miRNAs) are endogenous, noncoding small RNAs, approximately 19–25 nucleotides in length, which negatively regulate gene expression at the post-transcriptional level by targeting mRNAs for degradation or suppressing mRNA translation. They play a crucial role in many biological phenomena, including cell differentiation, proliferation and apoptosis, metabolism, and aging, thereby contributing to pathological processes in a variety of disorders [90]. Tissue factor expression and biologic function is modulated by several miRNAs [91], and decreased levels of miR-19b and miR-20a in monocytes from patients with APS have been identified as potential contributors to increased surface TF expression [92]. More recently, altered expression of miRNAs in patients with APS and SLE were found to be associated with atherothrombotic changes in leukocytes and endothelial cells, which could be further modulated by specific autoantibodies [93].

Next-Generation Sequencing to Identify Rare Genomic Variants

Genomic sequencing using massively parallel next-generation sequencing technologies is an effective alternative to locus-specific and gene panel tests to establish a genetic basis of disease [94]. Whole exome sequencing uses exon-specific oligonucleotides to enrich only protein coding sequences (representing approximately 30 million base pairs) that can be subsequently used for sequencing. This approach was used to identify a rare, disease-causing variant in the 3-prime repair exonuclease 1 gene (*TREX1*) in a 4-year-old girl with severe SLE [95]. This patient had a mildly elevated aCL IgM level, but was felt not to have APS. In a separate report, whole exome sequencing was used to investigate a patient with recurrent thromboembolic complications, and identified a mutation in *C3AR1* (complement component 3a receptor 1), suggesting that the patient might have an unusual presentation of atypical hemolytic uremic syndrome [96]. Whole genome sequencing expands the sequencing step to determine most, if not all, of the three billion DNA base pairs across the 46 chromosomes of an individual's genome. Using a next-generation sequencing approach to evaluate an extreme prothrombotic phenotype, e.g., catastrophic APS, may identify mutations associated with the more severe manifestations of APS.

A more complete picture of all genetic factors behind PAPS is needed to understand its pathogenesis and the differences and similarities between primary and secondary APS. The identification of genes will also allow us to begin with the difficult enterprise of understanding how genes affect cellular systems, research now ongoing in several laboratories.

Group Conclusions

The available data would support a genetic component to the development of aPL and APS, although this is a complex process. Acquired risk factors most likely also contribute to any thrombotic outcomes. Additional studies are necessary to identify and confirm any inherited aspect of this syndrome.

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Chapter 5
Mechanisms of Antiphospholipid Antibody-Mediated Thrombosis

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Introduction

The activity of antiphospholipid antibodies (aPL) is widely believed to be the driving force for pathological outcomes in the systemic autoimmune disease antiphospholipid syndrome (APS). Current consensus criteria identify the presence of anticardiolipin antibodies (aCL), anti-$\beta_2$glycoprotein-I antibodies (aβ_2GPI), and/or a positive functional lupus anticoagulant (LA) assay, together with clinical symptoms, to be the necessary elements for the classification of APS patients [1, 2]. However, there is burgeoning evidence for the clinical relevance of the less well-characterized "non-criteria" aPL, antiphosphatidylserine (aPS), antiphosphatidylserine-prothrombin (aPS/PT), anti-annexin A5, and anti-phosphatidylethanolamine (aPE) antibodies [2]. Persistent aPL high titers, in conjunction with typical thrombotic or obstetric manifestations, is the template for the diagnosis of APS patients, who can broadly be categorized into two groups: primary APS (PAPS) patients suffer from the disorder without a related connective tissue disease (CTD), or the disease can occur together with a concomitant CTD, most commonly systemic lupus erythematosus (SLE). Current evidence indicates very little difference with respect to clinical complications, the timing of those complications, and disease prognosis in these two groups, hinting at similar underlying pathogenic mechanisms [3, 4].

Both epidemiological and mechanistic studies inform our understanding of the thrombogenic capacity of aPL. Antiphospholipid antibodies are recognized risk factors for arterial and venous thromboembolism as well as for recurrent fetal loss in autoimmune patient populations; LA positivity is identified as the most important factor for thrombosis and pregnancy morbidity in APS patients [5, 6]. While the results of clinical studies provide strong evidence for the association of aPL with

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thrombosis, mechanistic in vitro and in vivo animal studies provide key insights into the underlying pathogenic mechanisms [7]. Currently available evidence makes clear that aPL have a broad range of activity, exerting effects on a variety of cells, coagulation factors, regulatory proteins, and inflammatory mediators. Indeed, the expansive effect aPL have on intracellular activity gives an indication of the complexity of their intermolecular interactions, which are both precipitated and propagated by inflammatory processes [7, 8]. Inflammation seems to play a central role in the production of aPL as well as the development of thrombotic or obstetric pathology in APS patients, and the inflammation-induced changes in the main antigen in APS, β_2GPI, seem to be particularly important [8–10].

Antiphospholipid antibodies are associated with clinically heterogenous thrombosis, ranging from mild to severe, even fatal, thrombosis, both venous and/or arterial [11–13]. Antiphospholipid antibodies upregulate hemostatic pathways through a variety of mechanisms that lead to a prothrombotic phenotype. The thrombotic risk is enhanced by additional individual factors; particularly important among these are common vascular risk factors, such as hypertension and hyperlipidemia, as these are modifiable [14]. That not all patients with aPL develop thrombosis supports a "two-hit" process, the first hit being the aPL-induced prothrombotic state and the second being exposure to prothrombotic situations such as surgery, immobilization, exogenous estrogen, or pregnancy [15]. There is no consensus on the mechanisms that contribute most to the development of APS manifestations. Major factors responsible for lack of agreement are heterogeneity in the source, character, and antigenic specificity of aPL studied, diagnostic criteria used to identify patients from whom antibodies were derived, and other design elements for studies evaluating aPL-induced mechanisms [16]. What does seem clear is that at least a subgroup of aPL drives disease pathology in APS.

In this chapter, we outline what is currently known of the mechanisms that contribute to the development of thromboemboli in APS, with particular reference to cell types, coagulation factors, and inflammatory mediators. We also review the facets of this topic for which there is no consensus among experts in the field, for instance, the relative importance of various mechanisms in the precipitation of thrombosis. Finally, we outline ongoing relevant studies, namely, those presented at the recent 15th International Congress on aPL (www.apsistanbul2016.org) and our vision of the future direction of APS thrombosis research.

What Is Known?

Thrombotic Risk Associated with Antiphospholipid Antibodies

Recurrent fetal loss and thromboemboli occur with high frequency in patients positive for aPL, with the greatest risk being attributed to LA [6, 17]. Antiphospholipid antibodies impart risk for both venous and arterial thrombi,

including deep vein thrombosis (DVT) with or without symptomatic pulmonary embolism (PE), myocardial infarction (MI), and strokes in individuals below the age of 50 [18, 19]. The APS Alliance for Clinical Trials and International Networking (APS ACTION) published estimates of aPL prevalence in various clinical groups, which were pregnancy morbidity (6% prevalence), DVT (10%), MI (11%), stroke (14%), and stroke in individuals less than 50 years (17%) [20, 21]. Although these estimates are based on systematic literature review and represent the best approximation to date, heterogeneity of study design, assay cut-off definitions, test reproducibility, and inclusion of varied criteria assays (especially in older studies) limit confidence in the veracity of these reports. Appropriately designed population studies with follow-up are therefore required.

A critical area of research in APS is to quantify the risk of thrombosis associated with aPL, to determine the need for specific therapeutic interventions. The presence of aCL or LA in SLE patients increases risk for venous thrombosis by factors of two and six, respectively, compared to normal populations [22]. In patients without an underlying autoimmune disease, aCL and LA increased risk for venous thrombosis by 1.5-fold and as much as tenfold, respectively [17, 23], and for arterial thrombosis by approximately threefold and fourfold, respectively [17]. Unfortunately, prospective studies evaluating the risks associated with criteria aPL are limited; best estimates come from the few available meta-analyses. Several studies included in these analyses are limited by small patient cohorts, lack of appropriate control populations, and variability in the types of aPL tested, the assays used, and the cutoffs selected to denote abnormality.

Experimental Thrombosis Models of Antiphospholipid Syndrome

The past two decades have seen numerous attempts to model APS in animals (Table 5.1). Experimental models demonstrated that aPL-accelerated thrombosis depends on inflammatory pathways and cellular mediators and extends beyond simple dysregulation of coagulation pathways. At least eight different models exist, which have many commonalities, but also unresolved discrepancies. For example, the Fc region of aPL is required in some studies [45], but not all [41, 43, 47]. The reader must therefore consider numerous factors, such as the source of aPL, the trigger to thrombosis, and the vascular bed under study.

Most extensively characterized is a model that involves administration of patient aPL to mice, followed by application of a standardized pinch injury to the femoral vein. A microscopic thrombus then forms and resolves over approximately 10 min, aPL-treated mice consistently form larger and more durable thrombi. The reproducibility and robustness of this model are an obvious advantage, leading to an impres-

Table 5.1 Experimental models of antiphospholipid antibody-mediated thrombosis

Model (order determined by date of first published report)	Notes on pathophysiology	Protective interventions
Femoral vein pinch injury (mouse): injection of patient aPL, followed by a standard pinch injury to the femoral vein. The same mice may also be characterized for vessel wall activation in carotid homogenates, distant from the site of thrombosis	Patient aPL promote larger and more durable thrombi after pinch injury [24], including IgG, IgM, and IgA preparations [25] Enhanced leukocyte adhesion in the cremaster microcirculation correlates with enhanced thrombosis in veins [26] Carotid artery homogenates express increased tissue factor in aPL-treated mice (albeit distant from the site of thrombosis) [27] Anti-β_2GPI isolated from leprosy patients are not thrombogenic [28] Affinity-purified antibodies specifically targeting domain I of β_2GPI are thrombogenic [29]	Protective drugs/inhibitors: Hydroxychloroquine [30] Anti-VCAM-1 [31] Fluvastatin [32] Anti-C5 [33] TIFI, a peptide that mimics domain V of β_2GPI [34] MG132, a specific NFκB inhibitor [35] Recombinant domain I of β_2GPI [36] C5-inhibitor rEV576 [37] Protective mutations: ICAM-1 [31] P-selectin [31] E-selectin [38] C3 [33] C5 [33] C5a receptor [39] TLR4 [27] Annexin A2 [40] ApoER2 [41] C6 [42]
Photochemically induced carotid thrombosis (hamster): injection of mouse monoclonal aβ_2GPI, followed by Rose Bengal-mediated photochemical injury	Mouse monoclonal aPL promote arterial thrombosis in a dose-responsive fashion [43]	F(ab′)2 aPL fragments can promote thrombus formation [43]
LPS-priming, with imaging of mesenteric microcirculation (rat): Coadministration of LPS and patient aPL, followed by intravital imaging of the mesenteric microcirculation for occlusions	This is essentially the only model in the literature that does *not* require vessel wall manipulation to induce thrombosis [44] Fibrin is deposited in the microcirculation when aPL and LPS are administered together or alone, neither is sufficient [44]	C6 deficiency and anti-C5 are both protective [44, 45] A functional Fc domain is required for an engineered monoclonal aβ_2GPI to induce thrombosis [45]

Laser-induced vessel wall injury in cremaster arterioles (mouse): injection of patient aPL or affinity-purified aβ_2GPI, followed by laser injury and intravital microscopy	aPL promote the rapid accumulation of both platelets and fibrin at the site of endothelial injury [46] aPL/β_2GPI complexes associate with platelets, more so than the endothelium, at the site of vessel wall injury [47]	Eptifibatide (an antiplatelet drug) blocks aPL-mediated thrombus formation, fibrin generation, and endothelial-cell activation [47] F(ab')2 aPL fragments can promote thrombus formation [47] A1-A1 (an antagonist of aPL-ApoER2 interactions) is protective [48]
Ferric chloride application to mesenteric microcirculation (mouse): injection of patient aPL followed by exteriorization of the mesenteric microcirculation and application of ferric chloride	aPL antagonize eNOS and thereby reduce leukocyte adhesion to the endothelium [49]	eNOS$^{-/-}$ and ApoER2$^{-/-}$ mice are protected from aPL-mediated thrombosis [49]
Dorsal skinfold chambers (mouse): injection of mouse monoclonal aPL, followed by laser injury to small veins (visualized in implanted chambers)	The authors argue that aPL trigger a prothrombotic state by inducing monocyte tissue factor, resulting in thrombosis at the site of laser-induced endothelial injury [50]	The NFκB specific inhibitor DHMEQ is protective [50]
Ferric chloride-induced carotid injury (mouse): injection of patient aPL or animal aβ_2GPI, followed by ferric chloride application to carotid artery (or femoral vein)	Studies show disparate results regarding endothelial activation. One suggests that aPL-mediated activation is predominately in the circulating compartment [51], while another presents evidence of endothelial activation (albeit it in aortas rather than the carotids themselves) [52]	aPL fail to accelerate carotid thrombosis in TLR4-deficient mice [51, 52]
IVC flow restriction (mouse): injection of cofactor-independent human monoclonal aPL followed by narrowing of the IVC to trigger thrombosis	Cofactor-independent aPL accelerate venous thrombosis The phenotype is not dependent on TLR4 [53] Acceleration of thrombosis by patient aPL is dependent upon neutrophils and neutrophil extracellular traps [54]	NOX2 mutation (in the circulating compartment only) is protective Anti-tissue factor is protective [53] Deoxyribonuclease and neutrophil depletion are protective [54]

aPL antiphospholipid antibodies, *ApoER2* apolipoprotein endothelial receptor 2, β_2*GPI* β_2 glycoprotein I, *C3* complement factor 3, *eNOS* endothelial nitric oxide synthase, *F(ab')2* divalent antibody fragment, *ICAM-1* intracellular adhesion molecule-1, *IgG* immunoglobulin G, *IVC* inferior vena cava, *LPS* lipopolysaccharide, *NFkB* nuclear factor kappa B, *NOX2* NADPH oxidase, *TLR4* toll-like receptor 4, *VCAM-1* vascular cell adhesion molecule-1

sive number of publications [24–42]. Disadvantages include, first, that it is debatable how well discrete mechanical injury to a vein wall mimics venous thrombosis pathogenesis in patients and, second, that vessel wall characterizations in this model typically are distant from the site of injury (e.g., studying tissue factor [TF] expression in the carotid artery, while assessing thrombosis in the femoral vein). However, the strengths of the model outweigh its weaknesses, as evidenced by the multiple pathways and concepts identified for further study (in some cases confirmed in independent models), including endothelium-leukocyte interactions, toll-like receptor (TLR) pathway signaling, complement cascade, nuclear factor kappa B (NFκB)-mediated transcription, and the key role of β_2GPI, among others. Manuscripts based on this model argue that activated endothelium is a critical regulator of aPL-associated thrombosis risk.

Newer models have moved from the venous circulation to the arterial, where explicit vessel wall damage is relevant. Some of these models use intravital microscopy, permitting the characterization of specific cells, such as platelets, as the earliest players in the thrombotic event [47]. Other recent studies argue for activation of circulating cells as the key contributor [51, 53], outweighing the contribution of endothelial activation. Two models deserve special note as they do not explicitly damage the endothelium but rely on either lipopolysaccharide (LPS) administration [44] or flow restriction [53] to activate the endothelium.

Cell Activation Is Key in Thrombotic Antiphospholipid Syndrome

Platelets

During the earliest days of APS research, investigators noted the frequent occurrence of thrombocytopenia in patients and animal models [55]. In patients, elevated urinary secretion of a major platelet-derived thromboxane metabolic breakdown product, 11-dehydro-thromboxane B2 (11-dehydro-TXB2), has been reported [56]. Thus in APS the link between thrombosis and platelet activation has long been suspected and was one of the first studied aspects of the disease [55]. Both epidemiological and mechanistic studies indicate that aPL induce expression of thromboxane B2 (TXB_2) and fibrinogen receptor glycoprotein IIb/IIIa (GPIIb/IIIa) in platelets, resulting in platelet aggregation [57, 58], and there is evidence for platelets' critical role in development of thrombosis in APS patients. Indeed, in an APS mouse model, B_2GPI/aB_2GPI complexes localize preferentially to platelets, inducing their activation at the site of arteriolar injury [47] and suggesting that at the level of microcirculation platelets may be primary targets of aPL and that their

activation leads to endothelial engagement and fibrin generation. A summary of mechanisms of aPL-mediated cell activation important for thrombus development in APS is shown in Fig. 5.1.

Endothelium

Given its constant interaction with whole blood, the endothelium has properties that potently counter coagulation/thrombosis [9]. The endothelium is the gateway by which inflammatory cells leave blood to enter tissue, a tightly regulated process that involves rolling, firm adhesion, and extravasation. These critical events are dependent upon selectin-mediated interactions that facilitate rolling and stronger integrin-mediated interactions that promote firm adhesion [59]. In animal models of aPL-accelerated thrombosis and in APS patients, there are signs of smoldering endothelial activation. For example, TF activity is increased in carotid homogenates from aPL-treated mice [27], a finding that correlates with increased leukocyte-endothelium interaction [26] and is supported by the facts that antagonizing E-selectin and P-selectin (key selectins expressed on endothelium) protects against thrombosis in mice and that strategies blocking the endothelial integrin ligands VCAM-1 and ICAM-1 do the same [31, 38]. Mechanistically, another study suggests that downregulation of endothelial nitric oxide synthase (eNOS) by aPL may also be an important factor increasing leukocyte-endothelium interplay [49].

In patients with APS, vascular endothelial growth factor (VEGF) and soluble TF circulate at increased levels, albeit without definitive evidence that these factors come from endothelium [60]. Kidney biopsies from patients with APS nephropathy suggest activation of the mammalian target of rapamycin (mTOR) pathway [61], which could enhance endothelial cell proliferation and certain types of APS-associated non-renal vasculopathy, if not thrombosis. Multiple studies demonstrate endothelium-derived microparticles in the circulation of patients with APS, as a possible surrogate for endothelial activation [62, 63]. Robust in vitro evidence indicates that aPL can activate endothelial cells to express TF and adhesion molecules [64, 65]. Mechanistically, NFκB, p38 mitogen-activated protein kinase (MAPK), and Krüppel-like factors (KLFs) have all been implicated in aPL-mediated activation of endothelial cells [66–68], demonstrating again ways in which aPL may co-opt pathways normally associated with more "authentic" inflammatory stimuli.

How does a primed endothelium contribute to thrombosis in patients? Most experimental models of APS trigger thrombosis by explicitly damaging endothelium using laser, ferric chloride, or pinch injury. In these cases studies looking at aPL/endothelium interplay should be interpreted with caution. The model of LPS-priming of the mesenteric microvasculature [44], a possible model of catastrophic APS, circumvents this issue and supports the concept that endothelial activation can trigger simultaneous and widespread thrombosis, at least in the microvascular compartment. However, one must remember that many clinical events are venous and

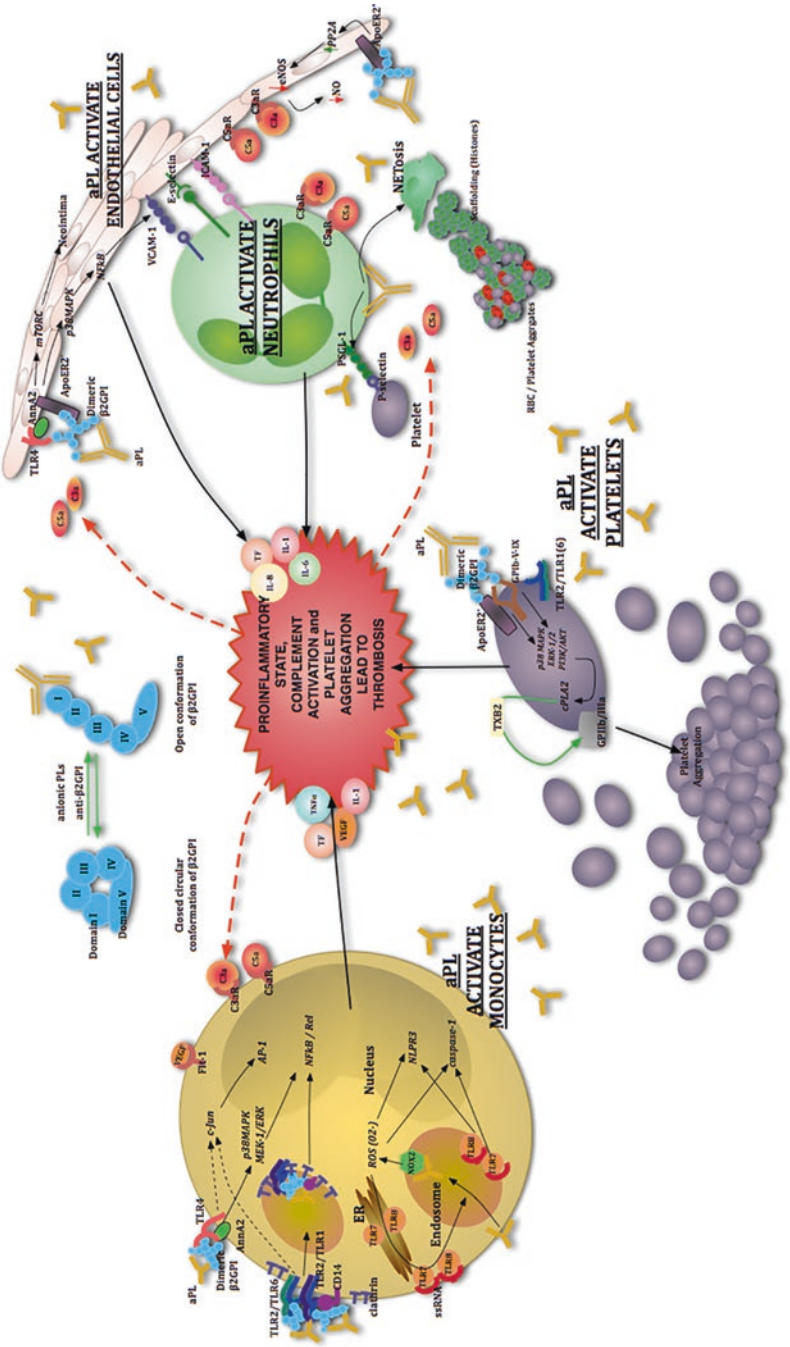

Fig. 5.1 Antiphospholipid antibody (*aPL*)-mediated cell activation: Pathogenic aPL activate endothelial cells (*ECs*) via *p38 mitogen-activated protein kinase* (*p38 MAPK*) and *nuclear factor κB* (*NFκB*) to produce several proinflammatory cytokines and adhesion molecules. Antiphospholipid antibodies can also

induce activation of the mammalian target of rapamycin (*mTORC*) pathway in endothelial cells to induce neointima formation (i.e., endothelial hyperplasia) commonly seen in aPL nephropathy and catastrophic APS. Signaling through apolipoprotein endothelial receptor 2 (*ApoER2*) antagonizes endothelial nitric oxide synthase (*eNOS*) resulting in reduced nitric oxide production. Antiphospholipid antibodies can also activate monocytes via several signaling pathway kinases and transcription factors including p38MAPK, MEK-1/ERK, NFκB/Rel, c-Jun, AP-1, Nox2, TLR7/8 and the NLPR3 inflammasome by engagement of various target receptors to produce proinflammatory cytokines. Signal activation can occur by engagement of receptors at the cell surface or through internalization with subsequent activation of intracellular receptors. Platelet activation occurs via engagement of ApoER2, GPIbα subunit of GPIb-V-IX and/or TLR2 inducing p38 MAPK, ERK-1/2, and PI3K/AKT to induce thromboxane B2 production, GPIIb/IIIa upregulation, and platelet aggregation. Infection/inflammation plays a central role in upregulating autoantibody production, causing endothelial injury and activating monocytes and neutrophils via complement-complement receptor interactions. Antiphospholipid antibodies can induce the formation of neutrophil extracellular traps (*NETs*) that propagate inflammation and contribute to thrombosis

highly localized, suggesting a confined breakdown of normal antithrombotic synergy between endothelium and blood. Experimental models that rely on more authentic thrombotic stimuli, and which characterize endothelium separately from the circulating cell compartment, will be required to resolve these questions.

Monocytes

Monocytes are likely to be important expressers of TF, especially in growing venous thrombi [69]. They are key players in the transition from innate to adaptive immune responses. Circulating monocytes have not been specifically analyzed in animal studies, beyond a recent manuscript stating that introduction of a NOX2 (NADPH oxidase) mutation into the circulating cell compartment (which includes monocytes and excludes endothelial cells) protects against venous thrombosis [53]. In that same study, an antibody targeting TF was also protective.

The ease of access to monocytes has led to their characterization beyond anything done with endothelial cells. For example, lupus patients with aPL have higher monocyte TF production than do lupus patients without, a fact known for 20 years [70], as do primary APS patients with thrombosis [71–73]. Monocytes from APS patients express higher levels of the proangiogenic cytokine VEGF and its receptor Flt-1 [74]. Gene profiling demonstrates upregulation of proinflammatory genes, including TLR8, CD14, and genes associated with oxidative stress [75, 76]. Highlighting the sometimes blurry intersection between coagulation and inflammation, APS monocytes upregulate certain protease-activated receptors (PARs) [77]. Several studies also demonstrate increased levels of monocyte-derived microparticles, a possible important source of TF [63], in circulation [78, 79]. In vitro, aPL trigger monocytes to express TF [80–83], and possibly other proinflammatory cytokines, such as TNF-α and IL-1β [68, 84, 85]. Going forward, it will be interesting to explore the role of monocytes in experimental models with intravital microscopy.

Neutrophils

Neutrophils have recently received attention as key perpetuators of arterial [86, 87], venous [69, 88], and microvascular thrombosis [89, 90]. Relevant to APS, mouse monoclonal aβ_2GPI activates neutrophils to release granules and produce hydrogen peroxide [91]. Neutrophils are activated in vitro by human monoclonal aPL [92] and by patient IgG [93, 94], with measurement endpoints that include expression of TF (similar to monocytes) [94], production of IL-8 [92], and release of prothrombotic neutrophil extracellular traps (NETs) [93]. While no consensus exists as to the signaling pathways that lead to neutrophil activation, roles have been suggested for surface β_2GPI [91], complement C5a [94], and TLR4 [92, 93]. Some [91], but not all [93], studies suggest a role for the Fc region of IgG. Only recently has attention turned to characterization of neutrophils from APS patients. Similar to

monocytes, APS neutrophils display altered mitochondrial membrane potential and evidence of oxidative stress, such as decreased intracellular glutathione [95]. Several groups are interested in the role of NETs in APS [93, 96, 97]. In the thrombosis field, NETs infiltrate arterial and venous thrombi [69, 86–88] and circulate at elevated levels in patients with microthrombosis [89, 90]. Neutrophil extracellular traps are tangles of chromatin and antimicrobial proteins extruded from neutrophils in response to both inflammation and infection [98]. Neutrophil extracellular traps activate platelets and the coagulation cascade and serve as scaffolding upon which a thrombus can assemble [99]; administration of DNase, which disassembles NETs, is protective in animal models of both arterial and venous thrombosis [100, 101]. Like lupus patients, those with primary APS have impaired ability to degrade NETs [96], and APS neutrophils have a lower threshold for NET release [93]. Like lupus patients, APS patients have elevated levels of circulating low-density granulocytes (LDGs) [102]; LDGs represent a subpopulation of neutrophils, of unknown origin, that release NETs in exaggerated fashion [97]. Neutrophils and NETs have only been characterized in one experiment model of APS [54]. Further studies will be needed to understand whether they play a role as initiators or at least perpetuators of the APS prothrombotic phenotype.

Target Receptors and Intracellular Signal Transduction

The activation of target cells by aPL is believed to occur through the interaction of aPL with the main antigen β_2GPI, often in dimeric form, and the binding of β_2GPI/aβ_2GPI complexes to cell surface and intracellular receptors [44]. The molecular mechanisms that lead to aPL-mediated activation of cell types important for thrombosis are summarized in Table 5.2. Many studies report the importance of aPL interaction with cell surface toll-like receptor 4 (TLR4) and annexin A2 (AnnA2) in the activation of ECs in APS [40, 103, 104]. Apolipoprotein endothelial receptor 2 (ApoER2) has also been implicated as a major target for engagement with β_2GPI/aβ_2GPI complexes on endothelial cells, platelets, and monocytes, most recently as a necessary factor for eNOS inhibition and nitric oxide (NO) downregulation by aPL [41, 49, 105]. Other putative cell surface receptors involved in aPL-mediated EC activation include TLR2, calreticulin, and nucleolin [103, 106].

The activation of the p38 MAPK pathway and subsequent nuclear translocation and activation of NFκB is a primary molecular mechanism for aPL activation of ECs [66, 107–109]. Nuclear factor kappa B is a cytoplasmic transcription factor complex that integrates inflammatory signals originating from activated pattern recognition receptors (interleukin-1 receptor/TLRs) and death domain-containing superfamily of cytokine receptors. Nuclear factor kappa B enters the nucleus upon stimulation where it induces the coordinated expression of approximately 5000 genes, the most rapidly inducible genes being those involved in intercellular inflammation, like *Gro*, *IL-8*, and *IL-6*, that may participate in thrombosis in APS [110, 111]. The involvement of the Ras-extracellular

Table 5.2 Molecular targets and signaling mechanisms in activated cells central to antiphospholipid-mediated thrombosis

Cell type	Receptors	Signaling pathways	Comments
Endothelial cell	Main: TLR4, AnnA2, ApoER2 Others: TLR2, calreticulin, nucleolin, complement receptors	*p38 MAPK* *mTOR* *Ras-ERK* *NFkB* *PP2A/eNOS*	Multicomponent protein receptor complex formation important in the initiation of cell signal. Activation results in intimal hyperplasia and production of proinflammatory mediators including NO, IL1, IL6, E-sel, ICAM-1, VCAM-1
Monocyte	Main: AnnA2, TLR4, TLR2(1/6) Others: TLR7&8, CD14, clathrin	*p38 MAPK* *MEK-1/ERK* *NFkB/Rel* *NLRP3* *NOX2* *c-Jun/AP-1*	Receptor complex formation in lipid rafts important for cell signaling. AnnA2 and TLR4 act in concert. TLR2 mediates aPL uptake into cell. Activation results in production of proinflammatory mediators, primarily TF
Platelet	GpIbα of GPIb-V-IX, ApoER2 Others: TLR2	*p38 MAPK* *ERK-1/2* *PI3K/Akt*	Both GpIbα and ApoER2 necessary for signaling. Activation results in TXB2 production and platelet aggregation. PF4 important for the spatial orientation of aPL when binding to platelet surface. Recent evidence suggests that platelet may be primary aPL target within the microvasculature
Neutrophil	Main: NETs, PSGL-1 Others: C receptors, TLR4, Fc-γ	*MyD88?* *IFN?*	Limited data available. Recent evidence suggests major role for aPL-induced NETs in inducing platelet activation and coagulation. PSGL-1 can accelerate aPL-mediated thrombosis. Proinflammatory phenotype in APS characterized by IFN-related genes, TLR signaling, and Fc-γ activation

AnnA2 annexin A2, *aPL* antiphospholipid antibody, *ApoER2* apolipoprotein endothelial receptor 2′, *c-Jun/AP-1* c-Jun/activator protein I, *eNOS* endothelial nitric oxide synthase, *E-sel* E-selectin, Fc-γ Fc-gamma, *GP* glycoprotein, *ICAM-1* intercellular adhesion molecule-1, *IFN* interferon, *IL* interleukin, *mTOR* mammalian target of rapamycin, *MyD88* myeloid differentiation primary response gene 88, *NET* neutrophil extracellular trap, *NFκB* nuclear factor kappa B, *NLRP3* nod-like receptor inflammasome, *NO* nitric oxide, *NOX2* NADPH-oxidase, *p38 MAPK* p38 mitogen-activated protein kinase, *PP2A* protein phosphatase 2A, *PF4* platelet factor 4, *PI3K/Akt* phosphatidylinositol 3-kinase/Akt, *PSGL-1* p-selectin glycoprotein ligand I, *Ras-ERK* Ras-extracellular signal-regulated kinase, *STAT3* signal transducer and activator of transcription 3, *TLR* toll-like receptor, *VCAM-1* vascular cell adhesion molecule-1

signal-regulated kinase (Ras-ERK) pathway in aPL-mediated endothelial cell activation was demonstrated using rat endothelial cells; however, the specificity of this activity to aPL is not confirmed as similar findings were obtained for cells stimulated by LPS and thrombin [112]. The phosphorylation of eNOS S1179 by

protein phosphatase 2A (PP2A) is attenuated through aPL interaction with β_2GPI and ApoER2 [49]. The mTOR pathway plays a role in vascular stenosis resulting from mechanical endothelial injury; it regulates cellular growth, proliferation, and survival through integration of a variety of signaling proteins. The development of vascular lesions in APS has been recently linked to activation of the mTOR pathway in response to aPL [61].

Annexin A2 and TLR4 are major cell surface targets for aPL-induced activation of monocytes in both venous and arterial thrombosis in APS [68], as has been the engagement of cell surface TLR2, in association with TLR1 and TLR6. Internalization of aPL in monocytes seems to occur via a clathrin- and CD14-dependent process [113]. Most studies indicate that activation of monocytes by aPL induces signaling in the p38 MAPK pathway, with simultaneous and independent activation of the MEK-1/ERK pathway, resulting in nuclear translocation and activation of NFκB/Rel proteins and proinflammatory gene activation [73, 74, 114]. A recent interesting series of experiments provides evidence that intracellular receptors TLR7 and TLR8 are targets of aPL in monocytes [53, 85]. These studies indicate that internalization of cofactor-independent aPL results in increased NOX2 and activation of the NLRP3 inflammasome and is important for inducing venous thrombosis in an APS mouse model. Another recent study shows that activation of the c-Jun/AP-1 pathway may also be important for aPL-induced arterial thrombosis [115].

On platelets, the main receptors that bind β_2GPI/aβ_2GPI complexes and induce platelet activation include ApoER2 and the glycoprotein Ibα (GPIbα) subunit of the GPIb-V-IX receptor [105, 116]. The p38 MAPK pathway is also important in aPL-mediated platelet activation; potential secondary roles of the ERK-1, ERK-2, and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathways have also been suggested [117–119]. Indeed, a mechanism of aPL-induced platelet activation involving the engagement of cell surface GPIbα and TLR2 and the subsequent action of PI3Kβ and α isoforms has recently been described [120]. Platelet factor 4 (PF4), a CXC chemokine secreted and bound by platelets, may also play an important role in platelet activation by stabilizing dimeric β_2GPI and facilitating its binding to aβ_2GPI and exposed phospholipids and receptors on the platelet surface [117].

Focused research on the role of neutrophils in APS has recently intensified; thus there are limited data on putative receptors and intracellular signaling pathways in APS. Most evidence suggests a major role for NETs in aPL-mediated thrombosis; in fact, DNAse treatment limits thrombus development in APS models [54].

Coagulation Pathways in Antiphospholipid Antibody-Mediated Thrombosis

Much evidence shows that aPL affect hemostasis at multiple levels. Potential mechanisms include activation of platelets, endothelium, monocytes and neutrophils, upregulation of coagulation, downregulation of fibrinolysis, and reciprocal

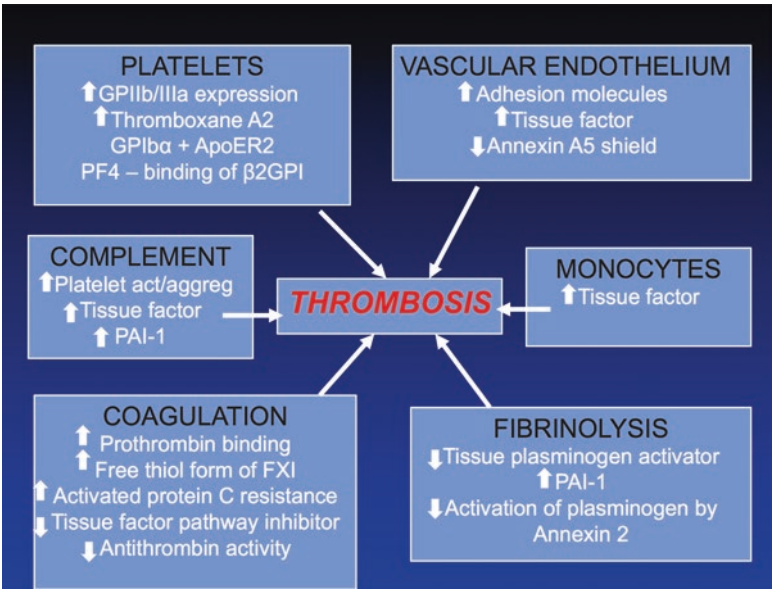

Fig. 5.2 Hemostatic abnormalities in antiphospholipid syndrome

activation of the complement and coagulation systems (Fig. 5.2). However, information is limited regarding the clinical importance and predictive value of these hemostatic changes.

Generation or exposure of TF at the site of trauma is the primary event that initiates clotting. Tissue factor functions as a cofactor for factor VIIa activation of factor X directly and indirectly via activation of factor IX. These coagulation interactions occur on negatively charged procoagulant phospholipids (mainly phosphatidylserine) on the platelet membrane, normally concealed on the inner platelet membrane and translocated in the intact platelet to the outside by membrane "flip-flop" [121]. Tissue factor pathway inhibitor (TFPI) is an important inhibitor of initiation of coagulation. Activation of the TF pathway is integral in the hypercoagulable state of APS, with upregulation of the TF pathway of coagulation [81, 122, 123], to which downregulation of TFPI is contributory [123–125]. Anti-β_2GPI suppresses TFPI-dependent inhibition of TF pathway coagulation [124].

The anticoagulant protein C pathway, which plays a central role in regulation of coagulation and maintenance of the fluidity of blood, may also be a key target for aPL. The physiological proteolytic activation of protein C by thrombin occurs on the vascular endothelium and involves two membrane receptors, thrombomodulin and endothelial protein C receptor (EPCR). Binding of thrombin to thrombomodulin shields the procoagulant exosite one of thrombin and facilitates protein C activation [126]. This reaction is intensified by the action of protein C on the endothelial surface by binding to EPCR. Protein C, along with its cofactor

protein S, exerts its anticoagulant effect by proteolytic inactivation of phospholipid-bound activated factor V, followed by inactivation of FVIII [127, 128]. Factor Va increases prothrombinase activity by approximately 10,000-fold, and its inactivation by activated protein C (APC) effectively prevents thrombin formation [126]. Although the most common antigenic targets of aPL are the phospholipid-binding proteins β_2GPI and prothrombin, protein C and protein S may also be important targets [129, 130]. In vitro aPL effects on the protein C pathway include inhibition of thrombomodulin-mediated activation and anticoagulant activity of APC [130]. Furthermore, aβ_2GPI binding to protein C can modulate its action [131] and its subsequent binding to phospholipid surfaces, thereby increasing thrombotic risk [132]. One mechanism proposed to explain how aPL initiate thrombosis is interference with APC's anticoagulant activity, resulting in acquired APC resistance (APCr) [133]. Thrombotic APS patients have greater APCr to both recombinant human APC and activation of endogenous protein C by ProTac suggesting that high-avidity anti-protein C antibodies, associated with greater APCr, may provide a marker for a severe thrombotic phenotype [134].

Complement activation has a pathogenic role in thrombotic APS [33, 135]. Limited in vitro data suggest that thrombin, factor Xa, and other serine proteases activate complement factors C3 and C5 to C3a and C5a, respectively, producing SC5b-9, the terminal complement component [136]. Complement activation reciprocally amplifies coagulation and inhibits fibrinolysis, through C5a, inducing expression of TF and plasminogen activator inhibitor 1 (PAI-1) [136]. Heparin has an anticomplement effect [137] through its inhibitory effect on thrombin and factor Xa, which cause activation of C3 and C5. Complement activation in APS may be modulated by rivaroxaban, a direct factor Xa inhibitor [138, 139], based on recent evidence that the activation markers C3a, C5a, SC5b-9, and Bb fragment were elevated in warfarin-anticoagulated thrombotic APS patients in the Rivaroxaban in APS (RAPS) trial at baseline [140]. However, C3a, C5a, and SC5b-9 (not Bb fragment) levels decreased in those who switched from warfarin to rivaroxaban, indicating that APS patients with previous VTE, on warfarin, have increased complement activation, likely via the classical pathway, that is decreased by rivaroxaban. Rivaroxaban may therefore provide benefit beyond anticoagulation by limiting complement activation [141].

The Role of Inflammation in Antiphospholipid Antibody-Mediated Thrombosis

Inflammation is rapidly mobilized in response to a variety of foreign and host-derived stimuli. Definitively distinguishing inflammatory response from coagulation is not always straightforward, given the multiple functions of key molecular and cellular components. For example, factor XII, the classic trigger of the contact activation

pathway of coagulation, plays an important role in activating inflammatory mediators like bradykinin [142]. Cellular actors like platelets also have multiple roles, from amplification of hemostatic pathways to sensing danger through innate receptors [143]. Investigators have increasingly turned attention to inflammation, as the field attempts to understand how APS targets diverse vascular beds and how those beds rapidly transition from clinically dormant to thrombosed. While alternative anticoagulants will be explored for therapeutic potential, breakthroughs in treatment will likely require understanding of the underlying inflammation.

β_2-Glycoprotein-I and Inflammation

β_2-Glycoprotein-I is a 326-amino acid glycoprotein, abundant in plasma, that can be deleted from both humans and mice without inducing an obvious phenotype [9]. Initially characterized as a lipid-binding protein [144] and noted to have five complement control protein (CCP) domains reminiscent of the complement regulator factor H [145], β2GPI is now thought to play a role in the innate immune system. Via its domain V, which is enriched with positively charged amino acids, β_2GPI binds negatively charged phospholipids such as phosphatidylserine. Given that such phospholipids are exposed on the cell surface as an "eat me" signal during apoptosis, investigators speculate that β_2GPI facilitates clearance of apoptotic cells. Indeed, β_2GPI binds to phosphatidylserine-expressing liposomes and cells in vitro [146], serving as a bridge to phagocytes [147]. Further, phosphatidylserine-containing liposomes are cleared more efficiently in mice when bound to β_2GPI [148]. While the specific receptors involved in clearance are not known, they are likely of the LDL receptor family [149].

The concept of β_2GPI as scavenger applies in other situations. β_2-Glycoprotein-I can partner with factor H to downregulate the alternative complement pathway [150]. Recent work shows that β_2GPI binds bacterial LPS to promote its neutralization and clearance [149]. β_2-Glycoprotein-I also functions as a sink for oxidative stress through its free thiol groups, with the oxidized form of β_2GPI (lacking free thiols) detected at high levels in APS patients compared to healthy subjects, other autoimmune disease patients, and thrombotic disease controls [151, 152]. Krilis et al. propose that oxidized-β_2GPI levels may serve as a biomarker of thrombotic risk, and they have developed an ELISA to measure posttranslational redox modifications of β_2GPI, including total β_2GPI and free thiol-β_2GPI. They also hypothesize that free thiol-β_2GPI format is protective in APS because thiol groups prevent hydrogen peroxide-induced cell injury; a decrease in free thiol groups via oxidation increases risk for oxidative stress-induced injury [153]. This topic is discussed in greater detail in Chap. 2.

β_2-Glycoprotein-I may play an active role in combatting infections, as neutrophil proteases cleave the full-length protein to generate antimicrobial cationic peptides [154]. β_2-Glycoprotein-I deficiency does not seem to be profoundly immunosuppressive, and the β_2GPI function(s) most important for host defense remain open to

debate. Nevertheless, this key APS antigen engages with the immune system and inflammation in multiple ways, setting the stage for a break in tolerance that leads to autoimmunity.

Complement System

"Complement" describes a system of circulating proteins that impact and activate each other via proteases, thereby promoting inflammatory cell recruitment, opsonization with pathogen clearance, and cell death. Often described as a cascade, the system can be activated by different stimuli with eventual convergence at the level of C5a generation (a chemotactic and proinflammatory protein) and assembly of the membrane attack complex (via components C5b, C6, C7, C8, and C9) [155]. The complement system plays a role in systemic lupus as a mediator of tissue damage [156].

The most compelling evidence implicating complement in APS comes from animal models. After early work showed that antagonizing complement protects against pregnancy loss [157], attention turned to complement's potential role in aPL-mediated thrombosis. In the femoral vein pinch injury model, targeting complement C3, C5, and C6 are all protective [33, 37, 39, 42]. Similarly, antagonizing C5 or C6 is protective in the LPS/mesenteric circulation model [44].

Given the antibody dependence of APS, it is tempting to speculate that activation of the complement system by the classical pathway (which is initiated by antibodies) is central to pathogenesis; however, F(ab')2 fragments or artificial β_2GPI dimers alone activate thrombosis in various models, arguing that the alternative pathway (spontaneous activation that gets amplified by pathogens or tissue damage that is not dependent on antibodies) may be the more important player [41, 43, 47]. This idea is supported by evidence of low-grade complement activation in APS [158–160] via the alternative pathway [161–163]. On the contrary, there are studies pointing to an association between aPL and classical pathway activation [135, 164, 165]. It is conceivable that aPL, β_2GPI (a regulator of factor H), and their complexes drive activation through both pathways. While the method by which complement activation might promote thrombosis is unclear, both vascular damage (via the membrane attack complex) and leukocyte recruitment (via C5a) are possibilities. A reason to probe these pathways is that complement inhibitors are likely to be increasingly used in clinical practice, with an example in APS-associated thrombosis being recently described [166].

Toll-like Receptors (TLRs)

Toll-like receptors evolved to recognize non-host molecular patterns that characterize pathogen invasion, the classic example being the recognition of LPS by surface-expressed TLR4. Displayed by leukocytes, TLRs rapidly trigger

proinflammatory cytokine release and cell activation, classic examples of innate immune function [167]. Toll-like receptor 4 is the only family member to be studied extensively in mouse models of APS, where its deletion protects against venous and arterial thrombosis [27, 51, 52]. Work with patient samples is limited; recent work suggests that APS monocytes are primed to express TLR2 and TLR4 on their surface when stimulated [168], while APS dendritic cells may overexpress endosomal TLR7 [169].

In vitro studies have probed the role of TLRs in mediating cell activation by aPL, especially aβ_2GPI, the rationale being the observation that some cell surface receptors for β_2GPI, such as annexin A2, do not have a cytoplasmic tail to mediate signaling. MyD88, an adapter protein that conveys signals from almost all surface TLRs (including TLR2 and TLR4), was identified more than a decade ago as a factor in aβ_2GPI-mediated activation of endothelial cells [170]. Subsequent studies implicated TLR4 as an aPL co-receptor on endothelial cells, including one that found a role for a complex consisting of annexin A2, TLR4, calreticulin, and nucleolin [103]. Toll-like receptor 4 has a role in the in vitro activation of monocytes [68, 84, 171, 172] and neutrophils [92, 93] by aPL. Whether LPS itself is a player in these pathways, either as a stimulator of TLR expression [92, 173, 174] or as a bridge between β_2GPI and TLRs [175, 176] is an area of ongoing investigation.

Some studies point to TLR2 as an aPL co-receptor [113], possibly excluding a role for TLR4 [106, 173]. Endosomal TLRs like TLR7 and TLR8 seem to be primed for hyperresponsiveness by aPL [169], creating a situation in which aPL amplifies production of proinflammatory cytokines such as IL-1β [85] or interferons. Because the vast majority of studies use in vitro systems and different types of aPL, it is difficult to create a cohesive model. Interestingly, β_2GPI depletion in male BXSB-Yaa mice, a mouse model of SLE dependent on duplication of TLR7, accelerates and potentiates the autoimmune phenotype implying that the main antigen in APS, β_2GPI, has a regulatory effect on TLR7 [177]. On balance the evidence supports a role for TLRs in APS, as mediators of cell activation and as key signaling molecules, which could tip an aPL-primed system toward thrombosis in response to environmental triggers.

What Is Controversial and/or Unknown?

Distinct Structural and Functional Characteristics of Antiphospholipid Antibody Types Related to Thrombosis

Experimental evidence implicates pathogenic aPL-induced activation of diverse cell surface receptors and intracellular pathways to promote thrombosis. This section will review whether the thrombogenic effects of these aPL can be distinguished by isotype, binding properties, and/or functional effects upon target cells.

Structural Characteristics

Strong evidence for pathogenicity relates to IgG binding the N-terminal domain (domain I or DI) of β_2GPI [36, 178], simultaneously cross-linking two β_2GPI molecules, activating biological pathways. There is emerging interest on the importance of other isotypes, particularly IgA aPL, in the pathogenesis of APS.

β_2-Glycoprotein-I contains five domains (DI-DV) and anchors to anionic PL via DV. Although antibodies directed against all domains have been reported, IgG anti-DI are most closely linked to APS and are elevated in patients with APS compared to disease and healthy controls [178–184]. Both affinity-purified IgG anti-DI from APS serum [29] and a human monoclonal anti-DI IgG aPL [10, 185] are prothrombotic in mice [186, 187]. In the same mouse model, recombinant human DI abrogates aPL-induced thrombosis [36]. A human monoclonal IgG anti-DI is, in two different animal models, prothrombotic and capable of causing fetal loss in naïve mice treated with LPS [45].

The significance of IgM aPL against DI is unclear; de Laat and colleagues reported that IgM anti-DI had no increase in their association with venous thrombosis compared to IgM aβ_2GPI [181]. Two studies reported that more than 50% of patients with IgA aβ_2GPI had reactivity against DIV-V of β_2GPI [188, 189]. Pericleous et al. [190] compared IgG, IgM and IgA aCL, and aβ_2GPI and anti-DI in patients with APS, SLE (no APS), and healthy controls; IgG aPL was the most common and highest-titer aPL, while IgA aβ_2GPI and anti-DI correlated more strongly with APS than did IgM. They also found that inclusion of IgG, IgM, or IgA anti-DI positivity increased the odds for APS detection compared to aCL and/or aβ_2GPI positivity alone; while IgG aCL, aβ_2GPI, anti-DI, and IgA anti-DI were associated with thrombotic but not obstetric complications.

Immunoglobulin A aCL are found in patients with SLE, many of whom do not have APS [191]. In contrast, isolated IgA aβ_2GPI positivity (in patients negative for IgG/IgM-aCL/aβ_2GPI and LA) is associated with thrombotic and obstetric manifestations of APS [192], and IgA aβ_2GPI are prothrombotic in mice [188]. For these reasons, published reviews propose testing for IgA (particularly aβ_2GPI) aPL only in IgG/IgM-aCL/aβ_2GPI and LA negative patients in whom APS is strongly suspected [193]. Readers should be aware of ongoing efforts to update laboratory and clinical classification criteria for APS highlighted at the recent 15th International Congress on Antiphospholipid Antibodies (discussed in Chap. 15).

Functional Characteristics

Cell surface receptors that interact with aPL and/or β_2GPI include annexin A2, ApoER2, and TLRs. Intracellular signaling through MAPK and the transcription regulator NFκB is demonstrable in aPL-mediated activation of target cells and is directly linked with TLR activation. Poulton et al. [194], systematically analyzed original studies on the effects of aPL on cell surface receptors and cell signaling

pathways and found evidence, from multiple approaches, that TLR4, p38 MAPK, and NFκB mediate pathogenic effects of aPL in thrombotic APS.

Heterogeneity among studies may derive from the fact that aPL in patients with thrombosis has different properties from aPL in patients with no thrombosis (obstetric APS). Few studies have compared effects on target cells of aPL from patients with and without thrombosis. In a series of papers, Lopez-Pedrera et al. [73, 74, 77] examined monocytes from patients with APS. They found differences in monocyte expression of p38 MAPK, NFκB signaling pathways, TF, VEGF, soluble Flt-1, and PAR1 and 2 in APS patients with thrombosis compared to APS patients with no thrombosis. Lambrianides et al. [171] found that IgG from these two APS patient clinical groups had different effects on p38 MAPK and NFκB activation in monocytes.

Lopez-Pedrera et al. [195] used traditional proteomics techniques to quantify 22 proteins in monocytes of 51 patients with APS (32 with thrombosis and 19 with pregnancy morbidity alone) and controls. Six proteins (annexin I, annexin II, protein disulfide isomerase, Nedd8, RhoA proteins, and Hsp60) most significantly altered, from monocytes of patients with thrombotic APS, were functionally related to induction of a procoagulant state and to autoimmune responses. They were subsequently found to be regulated by statin therapy [196]. Proteins implicated in recurrent spontaneous fetal loss such as fibrinogen and hemoglobin were also dysregulated in patients with obstetric APS.

More recently, Ripoll-Nunez et al. [197] used new proteomics techniques to analyze monocytes treated with IgG from 27 patients with different manifestations of APS. They found that four of the most significantly regulated proteins—vimentin, zinc finger CCH domain-containing protein 18, CAP Gly domain-containing linker protein 2, and myeloperoxidase—were differentially regulated in monocytes treated with thrombotic or obstetric APS IgG compared with healthy control IgG. They further characterized the proteome of thrombotic APS IgG-treated monocytes and found that many proteins identified possessed immune response, cytoskeletal, coagulation, and signal transduction functions relevant to APS. No single pathway is known to be dominant at this time.

Other studies demonstrate evidence for cytoskeletal protein involvement in endothelial activation in APS. Betapudi et al. [198] found that aβ_2GPI mediated induction of endothelial microparticle release depends on phosphorylation of the myosin regulatory light chain and assembly of actin-myosin networks. In addition, involvement of cytoskeletal proteins in aβ_2GPI-mediated activation of EC (via interactions with surface receptor annexin A2, which lacks a transmembrane domain) may explain how engagement of this receptor leads to activation of intracellular signaling pathways. Allen et al. [103] demonstrated that signaling through TLR4 is mediated through assembly of a multi-protein (annexin A2, TLR4, calreticulin, and nucleolin) signaling complex on the EC surface, so cytoskeletal protein may contribute to aPL-mediated EC activation and thus thrombosis.

Other studies show that aPL promote thrombosis by disruption of homeostatic and cytoprotective signaling in endothelial cells. An association between increased activity of the pro-survival mTOR pathway and endothelial hyperplasia in renal

biopsies has been reported in patients with APS compared to patients with lupus nephritis [61]. Other studies demonstrate aPL-mediated dysregulation of endothelial nitric oxide synthase (eNOS), which is responsible for maintenance of a healthy endothelium. Ramesh et al. [49] found that thrombotic-APS IgG inhibits eNOS activity in cultured human EC treated with VEGF, leading to inflammation and oxidation. Similarly, Ulrich et al. [199] found that the ability of thrombotic-APS IgG to antagonize in vivo and in vitro endothelial repair is mediated by eNOS deficiency.

Ramesh et al. [49] and Ulrich et al. [199], using ApoER2 null (−/−) mice, demonstrated a loss of APS IgG-mediated effects, thus implicating ApoER2 in aPL-mediated EC activation. Romay-Penebad et al. [41] directly studied involvement of this receptor in aPL-mediated thrombosis and found that the thrombogenic effects of a single IgG APS and of a constructed dimeric form of β_2GPI (that in vitro mimics β_2GPI-antibody immune complexes) were reduced in ApoER2 (−/−) compared to wild-type mice. Those effects induced by IgG APS and by the dimer were reduced by treatment of wild-type mice with soluble binding domain 1 of ApoER2 (sBD1), which inhibits binding of dimerized β_2GPI to ApoER2. Therefore, ApoER2 may also be involved in pathogenesis of APS thrombosis. This effect may not be specific to overt thrombosis since ApoER2 is required for the adverse effect of aPL on pregnancy outcomes in mice [200].

Different studies and methodological approaches demonstrate the importance of the cell surface receptors annexin A2, ApoER2, and TLR4 and the intracellular signaling pathways p38 MAPK and NFκB in mediating thrombotic effects of aPL. There is increasing evidence of the importance of IgA and anti-DI antibodies. Few studies have examined whether these structural and functional properties are specific to patients with thrombotic as opposed to obstetric APS. Further studies are required to determine whether these properties may help select potential therapeutic targets valid for all APS patients.

What Mechanisms Are Most Important for Thrombosis?

Many mechanisms have been described to explain the role of aPL in the induction of thrombosis (Fig. 5.3). Researchers have yet to identify and adequately characterize the mechanisms or groups of mechanisms that contribute most significantly to thromboembolism. While there is no consensus, there are patterns common to a number of mechanisms that provide insight. The general theory is that potentiation of thrombosis occurs as a result of a “two-hit” process, an initial priming phase in which a chronic proinflammatory state occurs in response to aPL activity followed by a development phase in which thrombosis is induced by an inciting factor, a hypothesis supported by the failure of thrombosis to develop in many patients with aPL and the inability of aPL infusions to induce thrombosis in APS animals models when given in isolation [24]. Indeed, thrombus development only occurs in these animal models when aPL infusions are coupled with a priming factor like LPS, chemical injury, or physical injury [43, 44, 49]. Another important consideration is

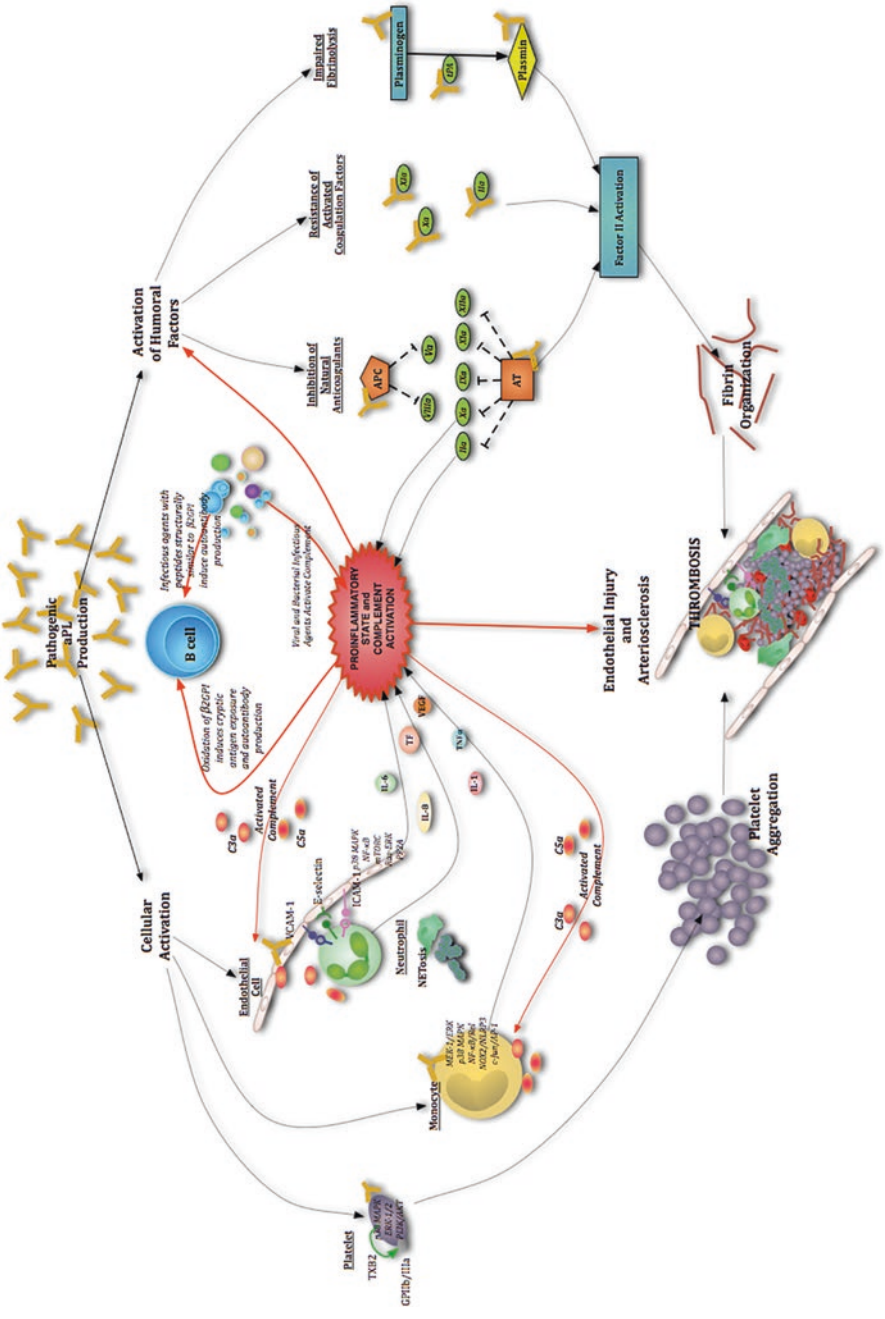

Fig. 5.3 Interaction of the various mechanisms by which antiphospholipid antibodies (*aPL*) induce thrombosis. Pathogenic aPL production occurs in APS patients most likely as a result of a break in tolerance induced by infectious agents with structural similarity to the main antigen β_2GPI. Oxidative stress leads

to the exposure of cryptic epitopes in β_2GPI, which results in further aPL production. Pathogenic aPL activate various cell types, namely, endothelial cells, monocytes, platelets, and neutrophils to produce proinflammatory cytokines resulting in a proinflammatory vascular phenotype. Pathogenic aPL also act on the coagulation pathway by directly acting on coagulation factors to increase their resistance to inactivation and by inhibiting fibrinolysis and natural anticoagulants like antithrombin and activated protein C with the ultimate outcome being increased thrombin activation and fibrin formation. Inflammation plays a central role as inflammatory mediators like complement C3a and C5a cause direct cell activation as well as activating coagulation factors which in turn can activate components of the complement pathway. Thrombosis occurs as a result of the synergistic activity of cell activation, a proinflammatory vascular environment, and inflammatory mediators causing endothelial injury, increased platelet aggregation, and fibrin organization

the fact that aPL may potentiate a pre-existing proinflammatory state (e.g., chronic infection); however, this has not been adequately addressed in research performed to date. At any rate, the full characterization of the underlying physiological changes inherent in the switch from priming phase to the development phase of aPL activity is a necessary step toward development of specific prophylactic or therapeutic agents for APS patients.

Interaction among receptors plays a key role in aPL-mediated activation of many cell types. The absence of an intracellular tail for the cell surface receptor AnnA2 led researchers to postulate the necessity of interaction between TLR4 and AnnA2 for aPL activation of intracellular signaling through this receptor. Subsequent studies confirmed that β_2GPI/aβ_2GPI complexes interact with AnnA2 and TLR4 in a manner dependent on lipid raft formation in monocytes as well as in a multi-protein complex including TLR4, AnnA2, nucleolin, and calreticulin on the EC surface [68, 113]. Similarly, the interaction of both ApoER2 and GPIbα receptors on platelets with β_2GPI/aβ_2GPI complexes is necessary for aPL-mediated platelet activation, while platelet factor 4 (PF4) is a necessary factor for binding of these molecules [105, 116, 117, 119]. Antiphospholipid antibodies also can induce activation of cytoskeletal proteins [103, 198]. Perhaps an initial indicator of the pathogenic ability of aPL, with respect to cell activation, is their capacity to induce the proper alignment and association of cell surface receptors, through their effect on cytoskeletal proteins, as a necessary step for inducing intracellular signaling.

The variable risk of thrombosis associated with different subtypes of aPL suggests that distinct antigenic determinants and/or functional capacities are linked to the potential for thrombosis. Compelling evidence exists that preferential aβ_2GPI recognition of domain I (versus other domains) is more likely to be associated with thrombosis [181]. The ability of aPL to limit coagulation reactions in vitro in a phospholipid-dependent manner (LA activity) seems to impart the greatest thrombotic risk [17, 23]. Lupus anticoagulant activity most likely occurs via one of the mechanisms that affect coagulation factors and/or their regulatory molecules; it seems to be linked to antibodies reactive against β_2GPI and prothrombin [201], suggesting that aPL-mediated mechanisms affecting coagulation pathways are particularly important for thrombus development. High-avidity antibodies with activity against protein C are associated with greater APC resistance and higher prevalence of severe thrombotic phenotype [134].

Recently published experiments debunk the belief that cofactor-independent aPL are nonpathogenic, since such aPL induce venous thrombosis in an animal model [53, 85]. A key finding of these studies is the internalization of cofactor-independent aPL, with engagement of endosomal TLR7/8 and NOX-dependent activation of NLPR3 inflammasome. These activating mechanisms occur independently of TLR2 or TLR4 engagement, in striking contrast to studies using cofactor-dependent aPL [27, 113]. It is possible that structural and functional characteristics of aPL speak directly to the combination of targeted receptors and to whether aPL act at the cell surface or are internalized to activate cell signaling. Variation in the intracellular signaling molecules activated could determine which genes are upregulated in response to aPL activity and, as a result, which variations in clinical APS phenotypes appear.

Antiphospholipid antibodies isolated from different APS patients have variable pathogenic capacity with respect to proinflammatory cytokine induction, promotion of thrombosis, inhibition of thrombus resolution, and increasing the adherence of leukocytes to ECs of microvasculature [26]. One study highlighted the preferential activation, by IgG aPL purified from thrombotic APS patients, of TLR4, p38 MAPK, and NFκB with subsequent TF production. This was not the case for aPL purified from patients with purely obstetric APS [171]. Another study reported that IgG purified from APS patients with obstetric pathology produced a more florid inflammatory response in trophoblast, characterized by increases in IL-8 and GRO-α, than did IgG from patients with purely thrombotic manifestations and negative controls [202]. Similarly, production of the antiangiogenic factor soluble endoglin (sEng) was increased in response to aPL from APS patients with isolated pregnancy morbidity, while another antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) was induced by aPL from APS patients with venous thrombosis [203]. Activation of the mTOR pathway by aPL leads to intimal hyperplasia in APS patients, suggesting a link between this molecular pathway and catastrophic APS and APS nephropathy [73].

Proulle et al. provide preliminary evidence that platelets are the primary targets for the β_2GPI/aβ_2GPI immune complex, with activation of ECs occurring subsequently [47]. This leads one to question if just one primary cellular target can account for all the reported consequences of aPL. Does aPL-mediated activation of each cell type occur in isolation or in an integrated manner? If activation is integrated, does it occur simultaneously or in an established sequence? Further studies are necessary. A key consideration is heterogeneity in the source, character, and antigenic specificity of aPL used to evaluate mechanisms as this may limit comparisons among studies [16]. Consensus on optimal methodology to study mechanisms will aid in determining which steps are essential for the development of clinical complications.

Current Research

Substantial work is being done to unravel the underlying mechanisms of aPL-induced thrombosis as evidenced by the numerous studies in this area presented at the 15th International Congress on Antiphospholipid Antibodies. A prospective cohort study finds that novel assays, like anti-DI and aPS/PT, as well as a Global Antiphospholipid Syndrome Score (GAPSS) above 16, best predict thrombosis in aPL-positive SLE patients [204]. A study investigating the relative clinical importance of antibodies against β_2GPI domain I versus antibodies targeting domain IV/V indicates that anti-DI antibodies are associated with thrombotic and obstetric manifestations, which was not the case for anti-DIV/V antibodies [205]. The importance of DI as a target for thrombosis development in APS was further highlighted by studies in an APS animal thrombotic model demonstrating the efficacy of a specific DI peptide in inhibiting the thrombotic effect of aPL infusions [206]. PEGylation of the DI peptide did not reduce its ability to inhibit aPL activity, a step forward in the development of therapeutic interventions specific for APS.

Immunoglobulin A/β_2GPI immune complexes (β_2A-CIC) in APS patients with isolated IgA aβ_2GPI positivity is strongly associated with recent thrombosis, levels dropping significantly after a 2-month period [207]. This finding seems to suggest that β_2A-CIC may serve as a marker for identifying IgA aβ_2GPI-positive patients at risk for thrombosis development. The same group provided preliminary evidence that IgG/β_2GPI (β_2G-CIC) and IgM/β_2GPI (β_2M-CIC) immune complexes were strongly associated with acute thrombotic events as well; however, further studies need to be done to determine their value as predictive markers of thrombosis [208]. In a separate study, based on the cell type investigated, PAPS and SLE patients had higher levels of cell-bound complement activation factor C4d compared to controls, indicating that cell-bound complement split products might help characterize clinical subtypes of APS and other autoimmune disease patients [209]. Wahl et al. presented a multicenter prospective cohort study investigating whether APC resistance, as determined by thrombin generation (TG), predicts thrombosis in aPL-positive patients with associated autoimmune diseases. The group, using multivariate analysis that includes typical risk factors like hypertension and previous thrombosis, found that APC resistance correlates with IgG anti-DI and IgG aPS/PT antibodies and is a significant predictor of thrombosis [210]. In a separate study, the non-criteria anti-TFPI and anti-protein C antibodies were associated with a severe thrombotic phenotype; however, this association was independent of a diagnosis of APS [211].

The neutrophil-lymphocyte ratio, which is associated with inflammatory changes in several diseases, was higher in both PAPS and another autoimmune disease-associated APS patients (compared to controls), but future studies will determine its suitability as a biomarker for thrombosis in APS [212]. A prediction model including mean platelet volumes and D-dimer levels identified thrombotic occurrence in a cross-sectional study of APS patients [213]. Loss of von Willebrand factor (vWF) multimer size regulation caused by severe ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) deficiency, either inherited or acquired (secondary to autoantibodies to ADAMTS13), is associated with the microvascular thrombotic disorder thrombotic thrombocytopenic purpura (TTP) [214]. ADAMTS13 autoantibodies and ADAMTS 13 dysfunction have been reported in APS [215]; however a case-control study indicates that while APS patients have elevated vWF and decreased ADAMTS13 levels, these changes do not correlate with thrombosis [216].

A mechanistic study that assesses β_2GPI and its biochemical posttranslational redox forms in a murine LPS systemic inflammation model provides evidence that the free thiol state of β_2GPI limits oxidative damage, supporting a model in which plasma β_2GPI increases in response to LPS (oxidative stress), which it binds and thereby limits TLR4 activation. These results have implications for TLR4-associated aPL mechanisms related to thrombosis development [217]. A comprehensive transcriptome analysis of neutrophils isolated from PAPS patients shows that these cells have a proinflammatory phenotype, which is characterized by overexpression of interferon-related genes, the TLR signaling pathway, Fc-γ receptors, and adhesion molecules including p-selectin glycoprotein ligand I (PSGL-1). In the same study,

PSGL-1 is upregulated in an independent cohort of 15 PAPS patients and is essential for acceleration of thrombosis in an APS murine model [218].

Another mechanistic study, using a separate APS murine thrombosis model, provides evidence that the adventitia primarily, but also the media, of the artery wall is the source of TF in aPL-mediated thrombosis; increases in thrombus size are associated with increases in carotid and macrophage TF [219]. Increased production of TF in arterial adventitia is common in atherosclerosis as well. Future studies will need to identify specific cellular sources of TF and the cellular dynamics in the arterial wall that occur in response to aPL. A study of circulating endothelial cells (CECs) in prospectively enrolled SLE and/or aPL-positive patients provides further evidence of this association [220]. Circulating endothelial cells are produced in response to endothelial injury; both venous and arterial thromboses are associated with elevated numbers of CECs.

Future Research Directions

Numerous targets of aPL have been identified, and the physiological changes that occur in response to antigen-antibody interactions that potentiate thrombosis have likewise been described. The main challenges over the next 10 years will be to identify mechanisms most important to the development of thrombosis in APS patients, whether or not the mechanisms that precipitate thrombosis vary among clinical phenotypes and, perhaps most importantly, the precise physiological changes in the vascular bed that result in the rapid transition from a proinflammatory state to thrombus development. A full characterization of these facets will be pivotal for developing effective agents that prevent the pathophysiological changes necessary for thrombus development in APS patients.

Preliminary data indicate that preferential, perhaps even exclusive, aPL-induced activation of cell signaling pathways occurs in APS patients with distinct clinical phenotypes and that this selectivity plays an undefined part in the development of varied clinical manifestations. To confirm these suspicions, carefully planned studies that focus on analyzing signaling pathways activated in target cells and the changes in coagulation factors, regulatory proteins, and inflammatory mediators need to be performed; such studies should use affinity-purified antibodies rather than whole IgG, IgM, or IgA preparations. Several experiments have used β_2GPI for affinity purification. Although it is a more challenging process, affinity purification is necessary to minimize non-specific effects in experimental models and to facilitate comparison of studies from independent laboratories.

Such antibodies should come from a large cohort of well-characterized APS patients, as well as disease and normal controls, followed over long periods of time. Researchers should keep in mind that the selectivity displayed by aPL may not be a matter of the presence or absence of activation of a signaling pathway or coagulation factor but may be far more intricate, involving variation in the intensity of activation of one pathway compared to another or variable assembly of and components within

receptor complexes, in lipid rafts on the cell surface, and on membranes of intracellular organelles. The end result could be subtle differences in the levels and combinations of prothrombotic and proinflammatory mediators. These differences, over the asymptomatic period and at the time of a triggering event, likely determine a patient's clinical presentation.

Going forward, it will be interesting to see whether animal models can align the studied vascular bed with pertinent physiologic triggers and at the same time incorporate real-time imaging to better understand the cells (endothelium and circulating cells) and pathways required for initiation (rather than propagation) of thrombosis. Platelets, endothelium, monocytes, neutrophils, coagulation factors, and inflammatory mediators may all be players that determine an APS phenotype. The question is whether they are equally important in all vascular beds and in all types of clinical events.

Group Conclusions

The goal of determining what underlying pathophysiological mechanisms and biomarkers are likely to define distinct APS clinical phenotypes is better APS patient characterization as a means to improve therapy. In clinical practice, if a defined constellation of clinical and laboratory parameters can be traced to activation of specific molecular pathways or molecular pathophysiological changes, that fact will facilitate targeted therapy tailored to a specific patient. Perhaps the most disheartening reality of APS research is that day-to-day management of these patients has remained unchanged over the past 30 years, focusing on conventional anticoagulation/antiplatelet treatment with low molecular weight heparin, low-dose aspirin (LDA), or warfarin singly or in combination [221, 222]. Consensus among experts in the field is that this approach is less than ideal.

Researchers, clinicians, and patients alike champion the development of novel treatments that target molecules and processes important in disease pathogenesis. In designing clinical trials, the appropriate characterization of specific clinical APS phenotypes that signal specific underlying molecular pathways will facilitate enrollment of patients most likely to benefit from the study drug. Indeed, a means of stratifying patients in terms of potential response will be helpful.

Regarding the clinical trials, several issues were highlighted in the APS Alliance for Clinical Trials and International Networking (APS ACTION) clinical trial designed to study the efficacy of hydroxychloroquine in preventing initial thrombosis in aPL-positive patients [223]. Common issues faced by clinical trials were highlighted by the 13th International Congress on aPL Clinical Research Task Force (Galveston, TX, USA [2010]) and by the 14th and 15th International Congress on aPL Treatment Trends Task Force (Rio de Janeiro, Brazil [2013], and Istanbul, Turkey, relocated to Cyprus [2016]). We invite readers to review their most recent report (Chap. 18). The future focus should be to develop diagnostic and therapeutic guidelines for what remains an inadequately managed disease.

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Chapter 6
Mechanisms of Antiphospholipid Antibody-Mediated Pregnancy Morbidity

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Introduction

Pregnancy complications are a frequent and unsolved condition in patients with antiphospholipid syndrome (APS). Presently about 80% of patients can deliver a live child, if managed properly before and during pregnancy. However, this does not mean that their pregnancies are without complications. Indeed, the risk of pre-eclampsia, intrauterine growth restriction (IUGR), and preterm delivery remains significantly increased in these women, despite drugs that are considered useful, such as heparin and low-dose aspirin. The explanation of our "clinical success" in this setting is partly due to the effect of the administered treatments, since they are able to reduce or prevent the complication occurrence, but it is also related to the higher attention that is paid to the patient throughout pregnancy. It is clear that to establish a proper timing for delivery, looking for the best compromise between fetal growth and fetal demise due to the deterioration of the maternal condition, or to have the prompt help of a neonatal intensive care unit, is today as necessary as, or even more than, an appropriate treatment. Low-dose aspirin and heparin are only "symptomatic" drugs in obstetrical APS, and "disease-modifying drugs" are still lacking. In light of this reality, every effort is necessary to better understand the biological basis of pregnancy complications observed in APS. Only through the comprehension of the fine pathogenic mechanisms, will we be able to apply treatments that are truly effective in removing the causes of pregnancy failure and complications in women with APS.

It is impressive to see how many mechanisms of damage to the fetomaternal unit have been linked to the pathogenic potential of antiphospholipid antibodies (aPL). The polyclonal nature of aPL may very well account for the existence of different antibody populations responsible for different pathogenic effects. But even in this

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setting, it is still difficult to understand to what extent fetal demise depends upon aPL-initiated inflammation and/or aPL-triggered modulation of trophoblast or endothelial cell function and whether antibody-mediated thrombosis may still be important. Only by careful analysis of these different hypotheses and the definitive identification of the primary pathogenic role of aPL will physicians be enabled to improve management of patients with obstetric APS.

The aim of this chapter is to review the known pathogenic mechanisms of pregnancy complications in APS and to provide a helpful instrument to all the workers in the field from both a clinical and basic perspective.

Animal Models of Antiphospholipid Antibody-Associated Pregnancy Complications

Experimental mouse models have been used to examine the mediators and mechanisms of aPL-induced pregnancy complications. Several groups have shown in mice that either immunization with the aPL antigen, β_2-glycoprotein I (β_2GPI) or the passive transfer of aPL promotes fetal resorption, fetal death, reduced litter sizes and IUGR [1–4]. Moreover, a study by Robertson et al. showed that while the passive transfer of human anti-β_2GPI antibodies (aβ_2GPI) to $\beta_2GPI^{+/+}$ mice triggered fetal loss, $\beta_2GPI^{-/-}$ mice were resistant to this antibody-induced effect, highlighting the importance of β_2GPI as a major targeting antigen in APS [5]. In these studies, low aPL doses (1–10 μg) were used, and transfer was mostly performed prior to mating or early in gestation. As such, there was the preexisting and consistent exposure to aPL throughout pregnancy, much like is seen in the true clinical scenario [1–4]. Together these in vivo models demonstrated a causative role of aPL in pregnancy morbidity. Impairment of maternal-fetal blood exchange because of intraplacental thrombosis was suggested to be the key pathogenic mechanism in aPL-mediated miscarriages. Placental thrombosis and infarction were in fact reported, and in vitro studies showed that aPL may induce a procoagulant state at the placental level through different mechanisms [6–10]. However, these observations were not supported by other studies, which failed to show intravascular or intervillous blood clots and histopathological findings suggestive for thrombosis in the majority of APS miscarriage samples and term placentae [6, 7]. This prompted investigation of alternative mechanisms of pathogenesis.

During embryo implantation and throughout gestation, highly regulated maternal immune responses allow the normal progression of pregnancy [11, 12]. Conversely, it is now widely accepted that acute inflammatory events at the maternal-fetal interface are responsible for a negative pregnancy outcome, and, as will be discussed below, pro-inflammatory mediators, such as complement, cytokines, and chemokines, have been shown to play a role in animal models of aPL-induced fetal loss (reviewed in [6, 7]).

Complement Activation as Mediator of Fetal Damage: Evidence from Mouse Models of Obstetric APS and Patients

In keeping with the observations of adverse pregnancy outcomes in earlier animal models of pregnancy failure in APS, more recent studies showed that intraperitoneal injections of human IgG with high titers of aPL activity in pregnant-naive mice after embryo implantation also resulted in fetal resorption and growth retardation [13–21]. For these studies, polyclonal IgG (10 mg) from APS patients with high titers aPL were passively transferred into mice on days 8 and 12 of pregnancy, and this resulted in a 40% frequency of fetal resorption compared to <10% in mice treated with IgG from healthy individuals and a 50% reduction in the average weight of surviving fetuses [17]. Pregnancy outcomes were similar in mice treated with monoclonal human aPL [17]. Using this model, it was determined that aPL localize to the placenta and their binding can directly stimulate effector cells. Classically, antigenic specificity localizes pathogenic antibodies, which, via their Fc domains, can then activate complement and/or crosslink Fc receptors expressed on effector cells. Fc receptors are not required for tissue injury in this APS model, because aPL induce miscarriage in mice lacking stimulatory Fc receptors [14, 22], although it is clear that ligation of Fc receptors may amplify the damage. Rather, in this passive transfer model, the complement system has been identified as critical for the pathogenic effects of aPL.

Complement activation has been shown to play an essential and causative role in pregnancy loss and fetal growth restriction [14, 17]. Blockade of the complement cascade in vivo with a C3 convertase inhibitor or deficiency of complement C3 prevented fetal loss and growth restriction in pregnant mice that were treated with human IgG containing aPL. Mice deficient in alternative and classical pathway complement components (factor B, C4, C3, and C5) and mice treated with inhibitors of complement activation (anti-C5 mAb, anti-factor B mAb, and C5a receptor antagonist peptide) were resistant to fetal injury induced by aPL [14, 17], indicating that both pathways contribute to damage (Fig. 6.1C). The complement component C5, and particularly its cleavage product C5a, was shown to be a key mediator of fetal injury: blockade of C5a–C5a receptor interactions prevented pregnancy complications (Fig. 6.2A). Indeed, the effectiveness of heparin, usually administered at sub-anticoagulant doses, may be, in part, because of its capacity to inhibit complement activation on the trophoblast. In this animal model, anticoagulation (with hirudin or fondaparinux), in and of itself, was not sufficient to prevent pregnancy complications in APS [15].

Because activated complement fragments have the capacity to bind and damage self-tissues, autologous bystander cells must be protected. To this end, most human and murine cells express soluble and membrane-bound molecules that limit the activation of various complement components at sited of inflammation [23]. Though activated complement components are present in normal placentas [24, 25], it appears that in successful pregnancy, uncontrolled complement activation is prevented by three regulatory proteins present on the trophoblast membrane: decay accelerating factor (DAF), membrane cofactor protein (MCP), and CD59 [26, 27]. All three pro-

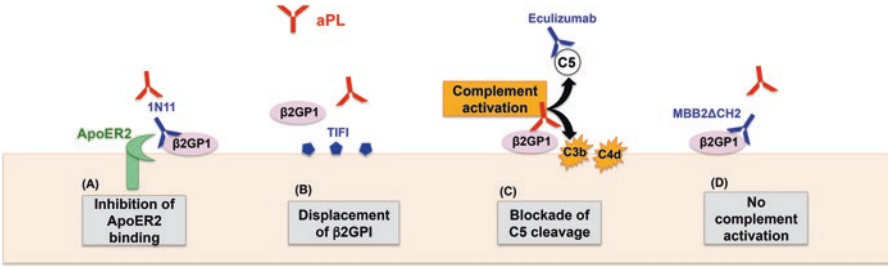

Fig. 6.1 Blocking the actions of antiphospholipid antibodies (aPL). (*A*) The monoclonal antibody, N11, prevents the actions of aPL on trophoblast and endothelial cells and attenuates aPL-associated pregnancy complications and thrombosis in mice. (*B*) The synthetic peptide TIF1 is a competitive blocker that displaces β_2GPI from the surface of the trophoblast and endothelium, thus preventing aPL binding and aPL-mediated thrombosis and pregnancy complications in vivo. (*C*) Eculizumab inhibits C5 cleavage and prevents pregnancy loss in experimental models. (*D*) The human monoclonal, MBB2ΔCH2, binds domain I of β_2GPI but fails to activate complement. MBB2ΔCH2 competes with patient aPL and prevents their pathogenic effect

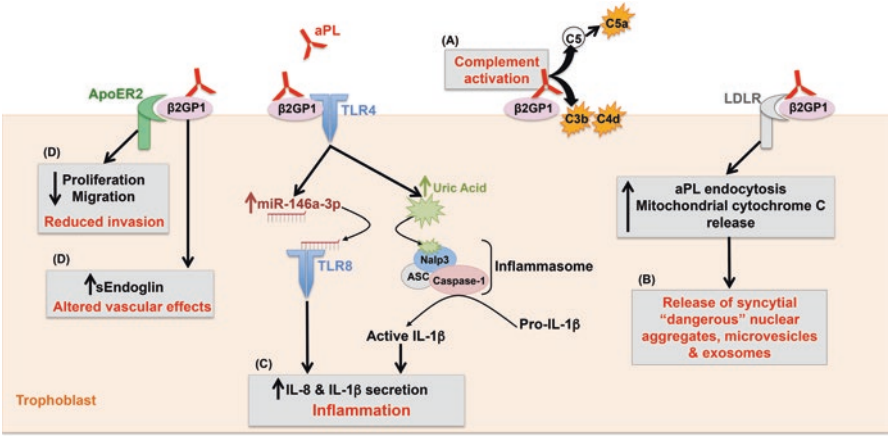

Fig. 6.2 Effect of antiphospholipid antibodies (aPL) on trophoblast cells. aPL recognizing β_2GPI expressed by the trophoblast: (*A*) activates complement on the cell surface; (*B*) become internalized via low-density lipoprotein receptor (LDLR) family members and in turn promote the deportation of "dangerous" syncytial nuclear aggregates and other microvesicles; (*C*) triggers secretion of inflammatory cytokines and chemokines by activating TLR and inflammasome pathways; and (*D*) promotes an anti-angiogenic profile and through ApoER2 reduces cell proliferation and migration

teins are strategically positioned on the trophoblast, along with circulating soluble regulators, and provide a mechanism to protect the fetus from damage due to activation of the complement pathway. Intact complement regulation is essential for maintenance of normal pregnancies, because in pregnant mice deficient in cell-bound

regulators of complement activation, fetuses die in utero surrounded by inflammatory cells and complement split products; breeding mice that lack complement inhibitors on a complement-deficient background rescues pregnancies [28, 29].

As discussed below, phosphatidylserine, externalized during trophoblast differentiation, allows β_2GPI to be expressed on the cell surface and, therefore, provides a target for aPL [30, 31], which can activate complement via the classical pathway to generate split products that mediate placental injury and cause fetal loss and growth restriction. The exaggerated complement activation that results may overwhelm the inhibitory capacity of local complement regulatory proteins allowing the complement cascade to proceed.

Studies in women support the role of complement in aPL-associated pregnancy complications. C4d is present in placentae from women with SLE and/or APS and from women with preeclampsia [32–34]. Indeed, in a systemic review literature of histology in placentae, one of the most common features in the placentae of aPL-positive women compared to control women was deposition of complement split product C4d [35] (Fig. 6.2A). Furthermore, in a prospective observational study in pregnant SLE/aPL patients, elevated levels of the alternative pathway complement activation product Bb are detectable early in pregnancy and are strongly associated with adverse pregnancy outcomes [36]. Mild hypocomplementemia has also been reported in primary APS in two studies [37, 38].

Observational studies in aPL pregnancies also emphasize the importance of dysregulation of the complement system in adverse pregnancy outcomes [39, 40]. Dysregulation can present as either excessive activation or inadequate regulation of this complex system. Soluble and membrane-bound complement regulatory proteins protect by limiting spontaneous alternative pathway activation. Indeed, defective function of complement regulators is associated with inflammatory and thrombotic injury associated with hemolytic uremic syndrome and glomerulonephritis [41, 42]. A report that 18% of 40 patients with SLE and/or aPL who had preeclampsia had heterozygous mutations in genes encoding three complement regulatory protein-membrane cofactor protein (MCP), complement factor I, some previously identified in atypical hemolytic uremic syndrome, a disease characterized by endothelial damage links complement activation to disease pathogenesis [43], underscores the role of complement in obstetric APS.

Inflammatory Mediators Downstream of Complement Activation as Effectors of Fetal Damage in Experimental Models of Antiphospholipid Syndrome

There are multiple effectors of fetal injury downstream of complement activation, triggered by C5a–C5a receptor interactions. Tumor necrosis factor (TNF)-α is one mediator that links complement activation and pathogenic Antiphospholipid antibodies to fetal damage. Antiphospholipid antibodies, specifically targeted to

decidual tissue, cause a rapid increase in decidual and systemic TNF-α levels. Complement 5-deficient mice were protected from fetal death and showed no increase in TNF-α levels identifying TNF-α as a critical intermediate that acts downstream of C5 activation [13]. That TNF-α is itself pathogenic is suggested by studies showing that miscarriage induced by aPL is less frequent in mice deficient in TNF-α or treated with TNF-α blockade [13]. Furthermore, in antibody-independent models of placental insufficiency and preeclampsia, complement activation at the maternal-fetal interface leads to elevation in local TNF-α levels, reduction of the essential angiogenic factor, vascular endothelial growth factor (VEGF), and, ultimately, abnormal placentation and fetal death. Blockade of complement activation or blockade of TNF-α improves spiral artery remodeling and rescues pregnancies, underscoring the relationship of these mediators and their importance [44].

Complement 5a also triggers fetal damage through induction of tissue factor (TF) expression. Treatment with aPL increases TF in neutrophils which enhances oxidative burst providing a mechanism for trophoblast injury and pregnancy loss triggered by these autoantibodies [19, 45]. Finally, complement activation products may cause an imbalance of angiogenic factors required for normal pregnancy. Satisfactory development of the fetomaternal vasculature is required for successful embryonic growth, and insufficient placental vascularization has been associated with early embryonic mortality, preeclampsia, and IUGR [46]. Normal placental development requires coordinated expression of angiogenic growth factors, and C5a–C5a receptor interactions trigger release of anti-angiogenic factors from leukocytes which can alter the balance of angiogenic factors in pregnancy and lead to the pregnancy complications associated with APS [16].

Antiphospholipid Antibody-Trophoblast Interactions

The placenta is a major target for aPL, in particular β_2GPI-dependent antibodies, which bind to human trophoblast. This may explain why pregnancy complications associated with placental development and function occur in women with APS. Indeed, the expression of β_2GPI on the placenta and, in particular, on trophoblast cell membranes is the prerequisite to explain aPL-placental tropism. While most cells will only bind β_2GPI on their cell surface under pathologic, stimulatory, or apoptotic conditions, when the inner negatively charged phospholipids become exposed onto the outer leaflet of the plasma membrane, the trophoblast is unusual in that it normally expresses these anionic phospholipids on its cell surface. This occurs as a result of the trophoblast's high level of proliferation and differentiation that is associated with tissue remodeling during placentation [47, 48]. As a result, the positively charged plasma protein, β_2GPI, can bind to phosphatidylserine exposed on the external cell membranes of trophoblast undergoing syncytium formation, although additional receptors may also be involved [6, 7, 49]. Furthermore, the trophoblast synthesizes its own β_2GPI, and this protein translocates to the cell surface [50]. In vivo, there is

evidence of β_2GPI localized to the surface of the extravillous trophoblast cells that invade the decidua and to the syncytiotrophoblast cells that are in direct contact with maternal blood [50, 51]. β_2-glycoprotein-I binds to the surface of the human trophoblast through the phospholipid-binding site in the fifth domain of the molecule, thus offering suitable epitopes for the maternal autoantibodies [6, 7, 49]. Hence, β_2GPI-dependent aPL appear to represent the main pathogenic autoantibodies in obstetrical APS. Accordingly, it has been hypothesized that most of them could be absorbed at the placental level (where β_2GPI is expressed) and not transferred to the fetus. This would explain why thrombotic events are rarely reported in babies born to aPL-positive mothers in spite of the high thrombophilic profile of neonates [52]. Although the syncytiotrophoblast and extravillous trophoblast populations both bind aPL recognizing β_2GPI, only the syncytiotrophoblast internalizes these antibodies via low-density lipoprotein receptors (LDLR) [53, 54]. The consequence of this aPL internalization is syncytiotrophoblast mitochondrial leak of cytochrome C [53] (Fig. 6.2B).

Since aPL bind to the trophoblast, it seems likely that the pathogenesis of pregnancy failure/complications in patients with APS is initiated at the placenta. Consequently, a number of studies have sought to evaluate the effects of aPL on trophoblast cells in vitro and have found that these autoantibodies affect several cell functions. Studies using human term trophoblast or choriocarcinoma cells show that aPL inhibit proliferation and syncytia formation [55–57], alter adhesion molecule expression [58], reduce invasiveness [56, 59–62], and decrease human chorionic gonadotropin [56, 61]. However, women with APS and pregnancy failure have circulating aPL at the time of implantation, and the most frequent clinical outcome is early pregnancy loss. Thus, more recent studies have shifted their focus to understanding the effects of aPL on the first trimester trophoblast. First trimester placental explants exposed to aPL have also been shown to produce less human chorionic gonadotropin (hCG) [63]. First and third trimester placental explants exposed to aPL generate distinct metabolic markers, ceramide, and diacylglycerols that may be involved in trophoblast responses to aPL [64]. Other studies using first trimester placental explants have found that the serum of patients with SLE/APS and recurrent pregnancy loss, anticoagulant-containing sera, or patient-derived aPL cause increased trophoblast apoptosis [63, 65, 66]. Anti-β_2GPI antibodies also augment the non-apoptotic shedding of trophoblastic material from first trimester placental explant cultures [67]. This is an important observation since during normal pregnancy, the placenta constitutively releases trophoblast microparticles, mononuclear trophoblast, and trophoblast syncytial knots from its outer syncytiotrophoblast layer into the maternal circulation [68], and in preeclampsia, a common outcome in APS-complicated pregnancies, shedding, or deportation of this material is significantly increased [69] (Fig. 6.2B).

Using in vitro cultures of human first trimester trophoblast cell lines and primary cells, mouse anti-β_2GPI monoclonal antibodies and purified patient-derived polyclonal aPL with β_2GPI reactivity have been found to enhance cytokine and chemokine secretion, specifically interleukin (IL)-8, IL-1β, growth-regulated protein (GRO)-α, and monocyte chemotactic protein (MCP)-1, which might explain the immune cell infiltra-

tion seen at the maternal-fetal interface. This aPL-induced inflammatory response is mediated by the Toll-like receptor 4 (TLR4)/MyD88 pathway [70], most likely because of molecular mimicry between β_2GPI and bacterial components, like lipopolysaccharide (LPS) [71, 72]. Indeed, in vivo studies have shown that animals immunized with microbial components develop aβ_2GPI antibodies and APS-like symptoms [73, 74]. Downstream of TLR4, trophoblast secretion of IL-1β secretion is mediated by the induction of endogenous uric acid, which in turn activates the Nod-like receptor (NLR), Nalp3, leading to Nalp3/ASC/caspase-1 inflammasome activation, and subsequent IL-1β [70, 75]. In parallel, IL-8 secretion is mediated by the induction of miR-146a-3p, which in turn activates the RNA sensor, TLR8 [76]. Thus, aPL-induced miR-146a-3p and uric acid act as endogenous secondary signals for trophoblast TLR8 and Nalp3 inflammasome activation, to drive trophoblast inflammation (Fig. 6.2C). The aPL-associated upregulation of trophoblast-derived uric acid and miR-146a-3p was found to be mirrored in the serum of women with aPL and adverse pregnancy outcomes [75, 76].

In parallel to the aPL-induced inflammatory response, aPL diminish the trophoblast's ability to migrate, independently of the TLR4 signaling pathway, by inhibiting the cell's constitutive production of IL-6, which in turn leads to decreased STAT3 activity [77], a critical mediator of trophoblast invasiveness [78]. This reduction in trophoblast migration was also found to involve aPL-induced upregulation of TIMP2 [79]. In a follow-up study, the surface-expressed receptor, apolipoprotein E receptor 2 (apoER2), was found to mediate this reduced migration [80]. However a recent study reported that patient-derived aPL reduce trophoblast invasion in a TLR4-depedent manner [81] (Fig. 6.2D).

Lastly, aβ_2GPI disrupts the basal trophoblast angiogenic factor balance, by inducing the secretion VEGF, PlGF, and sEndoglin levels (Fig. 6.2D). Although TLR4 is not involved in this response, functional MyD88 was required for the aPL-induced upregulation of PlGF, suggesting that receptors other than TLR4 that utilize MyD88, such as IL-1R or TLR8 [75, 76], are involved. All these aPL-mediated effects may play a role in causing a defective placentation [6, 7, 49]. Indeed, using an in vitro model of spiral artery transformation, aPL and sera from APS patients with pregnancy morbidity were shown to disrupt the normal trophoblast-endothelial cell interactions leading to reduced trophoblast-endothelial tube stability [82].

Data also suggest that aPL can cause abnormalities at the maternal side of the placenta. Impaired endometrial differentiation and lower expression of complement regulatory proteins (DAF/CD55) were found in endometrial biopsies from APS patients. These alterations before conception may compromise implantation and predispose to complement-mediated pregnancy failure [83, 84]. In addition, β_2GPI-dependent aPL are able to react with human stromal decidual cells in vitro and induce a pro-inflammatory phenotype [85]. These findings do suggest that APS-associated pregnancy complications can be mediated by several distinct pathogenic events.

At variance with the vascular manifestations of the syndrome, the two-hit hypothesis may not fit well with the APS obstetrical manifestations [6]. Passive infusion of IgG fractions with aPL activity induces fetal loss in naive pregnant mice and does not apparently require a second hit. β_2-glycoprotein-I is largely expressed in placental tissues even in physiological conditions [51, 86], and binding of labeled

exogenous β_2GPI infused into naïve pregnant mice to trophoblast and endothelial in the labyrinth was recently documented in vivo by eXplore Optix™ imager [87]. Thus, the large availability of the target antigen for pathogenic aPL at the placental level is in strong contrast with the lack of a comparable expression in other tissues of naïve mice and even in highly vascularized human tissues such as the kidney [7]. It is possible to speculate that a high expression of β_2GPI at the placental level, together with the hormonal and blood flow modifications linked to the pregnancy, is sufficient to favor the pathogenic activity of the autoantibodies without any additional factor.

Antiphospholipid Antibody Internalization into the Syncytiotrophoblast

Preeclampsia, a hypertensive disorder unique to human pregnancy, is one of the obstetric conditions often associated with aPL, and aPL have been reported to increase a woman's risk for developing preeclampsia almost tenfold [88–95]. While the pathogenic processes leading to preeclampsia are not fully understood, it is apparent that a toxin or toxins released from the placenta trigger maternal endothelial cell dysfunction. This maternal endothelial cell dysfunction is thought to be key to development of the maternal disease and may precede the onset of the clinical signs of preeclampsia by several weeks.

The human placenta is covered by a single multinucleated cell, the syncytiotrophoblast, which is bathed in maternal blood. Most cells produce subcellular lipid enclosed packages called extracellular vesicles that are released into the extracellular environment and are important in intercellular signaling. Extracellular vesicles from mononuclear cells are limited to microvesicles and nano-vesicles, some of which are exosomes. However due to its multinucleated structure, the syncytiotrophoblast also produces a unique type of multinucleated macro-vesicles called syncytial nuclear aggregates (SNAs), which are thought to be the end of the life cycle of the syncytiotrophoblast and therefore may be somewhat equivalent to apoptotic blebs produced by mononuclear cells. These multinucleated vesicles are larger than most mononuclear cells, being on average 72 μm in length and often have a teardropped shape [96, 97]. Syncytial nuclear aggregates are extruded from the surface of the syncytiotrophoblast directly into the maternal blood, which deports them to the maternal lungs where they become trapped in the pulmonary capillaries (the site where they were first identified in association with eclampsia over 120 years ago) [98]. While about 100,000 SNAs are deported daily from the placenta in all normal pregnancies [99], there is 20-fold increase in the number of SNAs deported in preeclampsia [96, 100, 101]. Syncytial nuclear aggregates are cleared from the maternal pulmonary capillaries on average in 3–4 days, and there is evidence that this clearance is due to phagocytosis of the SNAs by the maternal pulmonary endothelial cells against which they are trapped [102–104]. It has been suggested that SNAs from preeclamptic placentae may be one of the placental toxins that trigger preeclampsia [104, 105].

It has long been known that aPL can interact with the syncytiotrophoblast since, as described in the preceding section, aPL can reduce production of hCG, reduce formation of syncytiotrophoblast, and have been shown to disrupt the annexin V anticoagulant shield on the surface of the syncytiotrophoblast [10].

More recently it was reported that aPL, when incubated with first trimester placental explants, increases production of syncytial nuclear aggregates from the syncytiotrophoblast [103, 106]. So how do aPL induce the production of SNAs? Using a combination of murine monoclonal and patient-derived aPL, it was shown that aPL rapidly penetrate the syncytiotrophoblast with this process requiring as little as 2 min [53]. The internalization of the antibodies was receptor dependent. While the exact identity of the receptor remains unclear, transport of aPL into the syncytiotrophoblast was inhibited by receptor-associated protein (RAP), a low-density lipoprotein receptor (LDLR) family-binding protein (and by chloroquine) suggesting that aPL enter the syncytiotrophoblast via LDLR-mediated endocytosis [53] (Fig. 6.2B).

Immunofluor-gold electron microscopy was used to show that once internalized into the syncytiotrophoblast, the aPL were bound to mitochondria and that there was an increase in the number of mitochondria with swollen morphology associated with the aPL [53]. Further, once internalized, aPL disrupted mitochondrial function resulting in decreased oxidative phosphorylation though complex IV and the translocation of cytochrome C from the mitochondria to the cytoplasm of the syncytiotrophoblast [53]. This movement of cytochrome C has the potential to increase cell death in the syncytiotrophoblast by promoting the formation of the apoptosome, while the disruption of mitochondrial function may lead to a more necrotic type of death process in the syncytiotrophoblast. It is likely that, via these effects on the mitochondria, aPL increase the production of SNAs (Fig. 6.2B).

Not only do aPL increase the number of SNAs extruded from the placenta but importantly they change the nature of SNAs. It has been shown that SNAs from normal first trimester placenta, when phagocytosed by endothelial cells, render those endothelial cells resistant to activation, and this is important to the normal maternal adaptation to pregnancy [102]. In stark contrast, the SNAs extruded from placentae exposed to aPL become “dangerous” and induce endothelial cell activation similar to that seen in preeclampsia [104] (Fig. 6.3B). Providing further evidence that aPL induce the syncytiotrophoblast to extrude dangerous SNAs as a result of an interaction with the mitochondria, a proteomic comparison of SNAs from aPL or control antibody-treated placentae showed that of 72 regulated proteins 13 were involved in mitochondrial function [106].

It is not yet entirely clear what changes aPL induce in SNAs to make them dangerous, but it has been shown that the alarmin, high mobility group box 1 (HMGB1), is increased in the cytoplasm of SNAs from aPL-treated placentae. Similarly, the amount of “free” mitochondrial DNA, which is also an inflammatory danger signal, is increased in the smaller micro- and nano-vesicles that are released from aPL-treated placentae. Thus, aPL rapidly enter the syncytiotrophoblast via a receptor-mediated mechanism and disrupt mitochondrial function, resulting in the release of dangerous SNAs into the maternal blood. Maternal endothelial cells

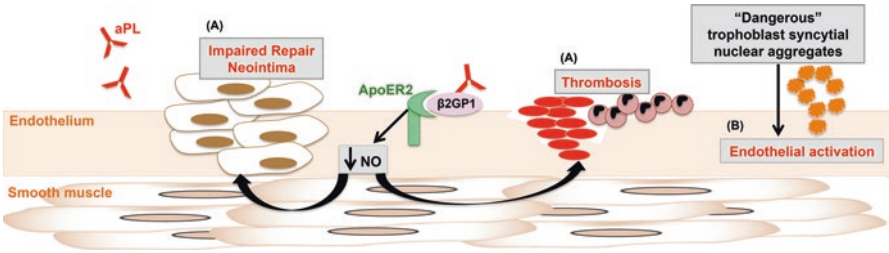

Fig. 6.3 Effect of aPL on the endothelium. (*A*) aPL-β_2GPI interaction with apoER2 impairs the production of nitric oxide (*NO*) leading to increased leucocyte adhesion, thrombosis, and neointima hyperplasia. (*B*) The "dangerous" syncytial nuclear aggregates extruded from placentae exposed to aPL induce endothelial cell activation

become activated in response to these dangerous SNAs in the same manner as occurs in preeclampsia. This provides a potential mechanism to explain why aPL are such a potent risk factor that predispose women to develop preeclampsia.

The Role of ApoER2 in Antiphospholipid Antibody-Mediated Placental and Endothelial Dysfunction

Apolipoprotein E receptor 2 (apoER2), also known as LRP8, is a member of the low-density lipoprotein (LDL) receptor family, which includes LDL receptor, LRP1, megalin, and very low-density lipoprotein receptor. When human apoER2 cDNA was first cloned, abundant expression of mRNA of the receptor was detected in the brain and in the placenta [107]. Subsequently, a critical role of the receptor was discovered in neuronal development as a receptor for reelin [108–110]. In the neuronal cells, reelin binding to apoER2 initiates activation of a series of kinases to regulate cellular function required for normal brain development [109–112]. More recently, it has been shown that the receptor is highly expressed in vascular cells, including platelets, monocytes/macrophages, vascular smooth muscle cells, and endothelial cells [113–120]. In 2003 de Groot's group reported for the first time that that apoER2, a splice variant of apoER2 expressed in platelets, can interact with dimerized β_2GPI in platelets [121]. Because aβ_2GPI-β_2GPI complex plays a key role in pathogenesis of APS, a series of studies ensued in in vitro experiment, in cell culture, and in mouse models to determine the role of apoER2 in the actions of aPL [119, 122–126].

In endothelial cells, our group (Chieko et al.) found that monoclonal aβ_2GPI or polyclonal human aPL attenuate activity of endothelial nitric oxide (eNOS) through dephosphorylation of the enzyme at Ser1177, the critical phosphorylation site for the activity of the enzyme, leading to impaired production of nitric oxide (NO) [119] (Fig. 6.3A), which is a key signaling molecule for maintenance of normal

vascular function. Nitric oxide produced by eNOS stimulates vascular relaxation, prevents endothelial inflammation, attenuates platelet activation, and reduces smooth muscle cell proliferation and migration [127, 128]. In cultured endothelial cells, we further demonstrated that inhibition of eNOS by aPL contributes to attenuated cell migration and increased adhesion to monocytes and that these aPL actions were abrogated with apoER2 knockdown by siRNA [119]. Mirroring the findings in culture, aPL administration in wild-type mice decreased eNOS-mediated vascular relaxation, impaired carotid artery reendothelialization after thermal injury, and increased leukocyte adhesion to the vascular endothelial layer [119, 129]. In contrast, apoER2-deficient mice were protected from these effects by aPL. A requirement for apoER2 in aPL-induced thrombus formation has also been determined in mouse models of APS by two laboratories independently [119, 126]. Our group has shown that aPL administration enhances thrombus formation in the mesenteric arterioles in wild-type mice, whereas it does not do so in apoER2 knockout mice [119]. Using eNOS knockout mice, we further demonstrated that eNOS antagonism is likely an underlying cause of aPL-induced thrombosis. The other group has also demonstrated that apoER2-deficient mice are protected from aPL- or dimerized β_2GPI-induced thrombosis in femoral vein [126]. The latter study also used soluble form of the apoER2 binding domain 1 (sBD1), a peptide mimicking the first ligand binding domain which blocks interactions with β_2GPI, to further verify that aPL-β_2GPI interaction with apoER2 mediates aPL actions in vivo. In addition to propensity for developing thrombosis, APS patients have elevated risk for non-thrombotic vascular occlusion [130–133] and greater stenosis of the celiac, intracranial, mesenteric, and renal arteries compared to non-APS individuals [134–140]. We have recently reported that when medial hypertrophy and neointima formation are invoked in mice by carotid artery endothelial denudation, aPL administration induces exaggerated neointima formation compared to control human IgG [129]. We further demonstrated that the neointima hyperplasia induced by aPL is related to impaired reendothelialization after injury. In contrast to the wild-type animals, apoER2-deficient mice were protected from the adverse effects of aPL on reendothelialization. Inhibitory effect of aPL on endothelial repair was prevented by concurrent administration of molsidomine, exogenous NO donor. These results indicate that aPL exacerbate both thrombosis and non-thrombotic neointima hyperplasia in the mouse models of APS and that the effect of aPL is caused by endothelial dysfunction mediated by aPL-β_2GPI interaction with apoER2 (Fig. 6.3A).

In the realm of pregnancy complications in the APS, numerous studies in trophoblast-derived cell lines, human primary trophoblasts, or placental explants have established that aPL treatment attenuates cell proliferation and migration, increases apoptosis, and impairs differentiation by altering the expression or activation of key proteins such as matrix metalloproteinases, interleukins, and chorionic gonadotropin [141]. Abundant expression of apoER2 mRNA was found in the placenta, and our recent study has indicated that both human and mouse trophoblasts express apoER2 protein [80, 107]. The study has further determined for the first time that apoER2 is required for aPL-induced trophoblast dysfunction in culture and fetal loss and IUGR in mice [80]. In cultured trophoblasts, the receptor was

required for aPL inhibition of epidermal growth factor (EGF)-induced Akt phosphorylation, cell migration, and proliferation. In mice, pregnant apoER2-deficient mice injected with human aPL showed attenuated fetal loss and IUGR compared to the wild-type mice. In addition to impaired trophoblast function, endothelial cell dysfunction in the placenta also influences pregnancy outcome through insufficient development of endometrial angiogenesis [142, 143]. It has been reported in cultured endometrial endothelial cells and in a mouse angiogenesis model that aPL impair endothelial cell migration and neovascularization [144]. The inhibitory action of aPL was associated with decreased expression of VEGF and matrix metalloproteinases, and it was abrogated by the synthetic peptide TIFI, a competitive blocker of aPL binding to endothelium [144, 145] (Fig. 6.1B). Our work showed in aortic or carotid artery endothelium that aPL inhibit endothelial cell migration, which is dependent on the presence of apoER2 [129]. The requirement for apoER2 in aPL-induced pregnancy loss has been shown in the mice with global apoER2 deficiency, and further studies are warranted to determine whether apoER2 in trophoblast, endothelium, or platelets (or in all three cell types) is specifically required for aPL-induced pregnancy morbidity. Interestingly, in humans polymorphisms in apoER2 gene have been associated with fetal growth restriction [146], although the role of the polymorphism in the pregnancy phenotypes in APS has not been explored.

In summary, aPL binding to β_2GPI activates apoER2 to disturb normal cellular functions in endothelial cells and trophoblasts, contributing to enhanced thrombosis and non-thrombotic vascular occlusion as well as adverse fetal outcome in mice. We have recently developed a monoclonal antibody, which inhibits aPL-induced formation of β_2GPI-apoER2 complex [147] (Fig. 6.1A). We have shown that the monoclonal antibody decreases thrombus formation and fetal loss in the mouse model of APS. Thus, apoER2 and its downstream effector molecules may provide a new mode of therapeutic interventions to combat APS.

Do Obstetric and Thrombotic Antiphospholipid Antibodies Have Differential Effects upon Target Cells?

What Lessons Have Been Learned from Observational Clinical Studies?

Current criteria tests used to identify persistently positive aPL in patients with APS do not predict specific APS manifestations [148]. Some patients with these aPL will develop only thrombosis; others only pregnancy morbidity, while some may not develop APS at all [149]. It is not clear why this discrepancy occurs, and one way in which to interpret these findings is that aPL from patients with obstetric APS may bind different antigens and cellular receptors to affect tissues of the body in different ways to aPL from patients with thrombotic APS.

Historically, pregnancy complications in patients with APS were considered to be due to thrombotic events at the maternal-fetal interface. Histological comparison, however, of products of conception from aPL-positive and aPL-negative patients with recurrent early miscarriage has shown a specific defect in decidual endovascular trophoblast invasion in patients with APS [150] and that placental infarction is not specific to patients with APS [151]. Increasing evidence (reviewed above) shows aPL to have inflammatory effects on endometrial and trophoblast cells resulting in impaired implantation and placental development.

Cohort studies show that some patients with APS experience only thrombosis or only pregnancy morbidity but not both. In a European study of 1000 unselected patients with definite APS [152], there were 820 female patients all of reproductive age. Of these patients, 590 experienced obstetric APS [153] and 230 (28% of the entire female cohort) experienced thrombotic APS alone. During a 10-year follow-up period, the most frequent manifestation was thrombosis with thrombotic events appearing in 166 (16.6%) patients during the first 5-year period and in 118 (15.3%) patients during the second period. In the same 10-year period, 127 (15.5%) women became pregnant with a total of 188 pregnancies and 137 live births [154]. The most frequent obstetric complication was early (<10 weeks) pregnancy loss in 16.5% of pregnancies. Interestingly, only three out of 121 (2.5%) women with pure obstetric APS manifestations at study onset developed a new thrombotic event during the first 5-year study period [149].

To enable a detailed analysis of long-term clinical and laboratory characteristics of patients with pure obstetric APS, a European registry has been set up and reported 5-year follow-up data from 247 patients fulfilling only obstetric APS classification criteria at recruitment [155]. Despite further obstetric complications reported in 129/247 (52.2%) patients, there were very few venous – gestational (1.21%) and puerperal (6.7%) – thrombotic events and only three postpartum arterial thrombotic events, two coinciding with abrupt cessation of heparin and aspirin.

Are There Distinguishing Binding Properties of Antiphospholipid Antibodies in Obstetric Antiphospholipid Syndrome?

Many studies have sought to determine whether specific aPL binding properties are associated with thrombotic or obstetric manifestations. The largest study of adverse pregnancy outcomes in SLE is the PROMISSE study. This prospective multicenter study has assessed the frequency of adverse pregnancy outcomes and clinical and laboratory variables that predict them, in women with aPL and/or SLE at conception. To date this study has reported that LA, rather than aCL or aβ_2GPI, positivity is the primary predictor of adverse pregnancy outcomes in two independent groups ($n = 144$ and $n = 44$) of aPL-positive patients [156, 157].

The association, however, of specific aPL with particular clinical manifestations in established APS cohorts is less certain. The Euro-APS and Euro-obstetric APS

studies did not find that specific criteria aPL distinguished thrombotic or obstetric APS. In fact, the Euro-obstetric APS cohort found identification of all three criteria aPL individually or in different combinations to be important in identifying obstetric APS [155]. Overall, no one criteria aPL has emerged as the leading predictor of thrombotic or obstetric manifestations in patients with APS.

It is not clear whether antibodies directed against non-criteria aPL may be more specific for pregnancy loss APS. A recent systematic review was unable to examine whether any non-criteria aPL evaluated (including anti-DI and various IgA aPL) were more frequent in particular APS features because none of the selected studies gave a breakdown of this information and only reported overall prevalence figures for APS [158]. A recent multicenter cohort study [159] that measured IgG, IgM and IgA aCL, aβ_2GPI, and anti-DI in APS and controls found IgG aPL to be the commonest and highest titer aPL, while IgA aβ_2GPI and anti-DI correlated more strongly with APS compared to IgM counterparts. IgG aCL, aβ_2GPI, anti-DI, and IgA anti-DI were associated with thrombotic, but not obstetric complications in patients with APS. Therefore, a convincing association between non-criteria aPL and specific APS manifestations also remains to be proven.

Are There Differences in the Cellular Effects of Obstetric and Thrombotic Antiphospholipid Antibodies?

Experimental evidence presented throughout this chapter highlights how pathogenic aPL interact with β_2GPI and/or different cell surface receptors to activate inflammatory pathways leading to the manifestation of obstetric APS. Few studies, however, have specifically set out to compare effects of aPL from patients with and without thrombosis on target cells. Lopez-Pedrera et al. compared monocytes extracted from blood of ($n = 44–62$) patients with APS with healthy control (HC) monocytes and found differences in p38MAPK and NFκB signaling pathways as well as tissue factor, VEGF, sFlt-1, and PAR1 and PAR2 expression [160–162] in monocytes from APS patients with thrombosis than in monocytes from APS patients with no thrombosis. Lambrianides et al. [163] found that IgG from these two clinical groups in ($n = 27$) patients with APS had different effects on p38MAPK and NFκB activation in monocytes.

Similarly, few studies have compared the effects of thrombotic versus non-thrombotic APS-IgG in cell types relevant to obstetric APS. Mulla et al. [70] demonstrated that IgG purified from patients with obstetric APS stimulated trophoblast production of IL-8 and GRO-α significantly more than IgG from patients with thrombotic APS [70]. Immunoglobulin G purified from patients with obstetric APS triggered significantly more sEndoglin secretion than IgG from patients with thrombotic APS, while thrombotic aPL induced a greater trophoblast VEGF and sFlt-1 response in the than obstetric aPL [164]. Another comparative study found that only IgG from patients with pure obstetric APS inhibit in vitro trophoblast invasion in a TLR4-dependent manner, compared with thrombotic APS-IgG which lacked this effect [81].

Other studies have utilized proteomics to identify novel pathways in different manifestations of APS. Lopez-Pedrera et al. [165] using traditional proteomics techniques identified 22 proteins that were altered significantly in monocytes of patients with APS (32 with thrombosis and 19 with pregnancy morbidity alone) compared to controls. They found proteins implicated in recurrent spontaneous abortion to be significantly dysregulated in patients with obstetric APS, while six proteins that were most significantly altered among monocytes from patients with thrombotic APS were all functionally related to the induction of a procoagulant state. Ripoll-Nunez et al. [166] utilized newer proteomics techniques to analyze human monocytes treated with IgG from ($n = 27$) patients with different manifestations of the APS. They found that four of the most significantly regulated proteins were differentially regulated in monocytes treated with thrombotic or obstetric APS-IgG, compared with HC-IgG, thus providing further evidence that the monocyte proteome is differentially regulated by obstetric compared with thrombotic APS-IgG.

As discussed, engagement of the apolipoprotein E receptor 2 (apoER2) has been shown to be required for the adverse effect of aPL on pregnancy outcomes in mice [80]. This effect, however, is not specific to obstetric APS since ApoER2 has also been implicated in aPL-mediated endothelial cell activation [119, 129] and aPL-mediated thrombosis in mice [126].

In summary, evidence is emerging from a variety of clinical and laboratory studies to support the hypothesis that obstetric APS may be distinguished from thrombotic APS by more than just the initial pattern of clinical manifestations. In particular, long-term follow-up reveals little overlap between obstetric and thrombotic manifestations in patients with obstetric APS. Furthermore, there are demonstrable differences in the functional effects of obstetric compared with thrombotic APS-IgG in vitro upon various relevant target cells. Further in vivo work, however, is required to fully answer the question of whether obstetric, and thrombotic aPL have different effects on target cells in patients with APS.

Targeting β_2-Glycoprotein I to Prevent Obstetric Antiphospholipid Syndrome

There is sound evidence that β_2GPI is present both at the maternal (decidual and uterine endothelial cells) and fetal side (trophoblast cells) acting as an antigenic target for β_2GPI-dependent pathogenic aPL in obstetric APS [6, 167].

β_2-glycoprotein-I is a cationic plasma protein, and it was suggested to bind to phosphatidylserine exposed on the external cell membranes of trophoblast undergoing syncytium formation, but additional receptors may also be involved [6]. β_2-glycoprotein-I binds human trophoblast and endothelium through the phospholipid-binding site in the fifth domain of the molecule, making the immunodominant domain 1 (D1) epitope available as shown by the in vitro reactivity with MBB2, a human monoclonal aβ_2GPI that recognizes D1 [168, 169].

While the in vivo presence of β_2GPI in vascular tissues cannot be detected under resting conditions, but only after an inflammatory stimulus (two-hit theory), this apparently is not the case for uterine endothelium [6, 170]. Initial ex vivo observations documented the presence of the protein on villous trophoblast of human term placentae using direct immunofluorescence. Recently, in vivo imaging studies provided direct evidence for the binding of β_2GPI to uterine endothelium and to trophoblast at the implantation sites in pregnant mice. The current view holds that β_2GPI binds to anionic phospholipids exposed (or to the other receptors) on the cell surface and undergoes a conformational change that permits binding of the antibodies to a cryptic epitope (e.g., D1) resulting in dimerization of the antigen and stabilization of the complex. Alternatively, the adhesion of β_2GPI to the cell surface may increase the antigen density thereby favoring the binding of low avidity autoimmune aPL. The implication is that the affinity of β_2GPI for surface anionic phospholipids/membrane receptors is relatively low in the absence of antibodies. This does not seem to be the case since the in vivo model shows a stable binding of the molecule. The reason for such selective binding is not clear, but a local hormonal environment and/or the physiological changes related to pregnancy may play a role.

The induction of circulating aβ_2GPI in mice mimics the situation in human APS, but it does not change the tissue distribution pattern of the molecule. However, the antibodies contributed to mediate fetal resorption similar to that obtained by passive transfer of human aPL in pregnant mice [170]. Local activation of the complement system is involved as supported by the protective effect of complement inhibition or deficiency [13–19, 169, 170]. Hence, taking into account the pathogenic role of the antibody binding to tissue β_2GPI and the consequent complement activation, inhibiting/reducing these two critical steps appears a rational strategy for preventing obstetric APS.

Heparin and low-dose aspirin are widely accepted to be effective in preventing aPL-associated pregnancy complications, but their actual pharmacological mechanism(s) of action is still a matter of research. However, in vitro models showed that β_2GPI binds heparin with higher avidity than tissues [62]. Hence, heparin can bind and displace β_2GPI from the tissues making the molecule no longer available for pathogenic aPL. Although such an effect is only supported by in vitro models, it is in line with the theoretical targeted strategies in obstetric APS, e.g., inhibiting or reducing the presence of β_2GPI on the uterine and placental tissues.

The synthetic peptide TIF1 spans Thr101-Thr120 of ULB0-HCMVA from human cytomegalovirus and shares a similar sequence with the β_2GPI phospholipid-binding site. The peptide prevents aPL-mediated thrombosis in vivo and inhibits the in vitro binding of labeled β_2GPI to human endothelial cells and mouse monocytes. As aPL do not react with TIFI, its protective effect was thought to result from the ability to compete with the phospholipid-binding site of β_2GPI, displacing the molecule from the cell surfaces and thus inhibiting aPL binding [171]. We showed that TIFI, unlike an irrelevant peptide, inhibits the in vitro reactivity of aβ_2GPI monoclonal antibodies with human trophoblast monolayers, suggesting a comparable displacing effect [145]. In line with our working hypothesis, repeated infusions of TIFI in pregnant-naive mice was able to protect them from fetal resorption and growth retardation induced by human β_2GPI-dependent aPL IgG [172] (Fig. 6.1B).

Complement inhibition may represent another therapeutic approach owing to its key role documented in animal models. Several complement inhibitors or blocking antibodies are now available, but their use in human pregnancy is limited by safety reasons. We recently reported an alternative strategy showing that a non-complement fixing aβ_2GPI human monoclonal antibody is protective both in a vascular and obstetric APS model. A recombinant antibody recognizing D1 domain of β_2GPI (MBB2) was generated that induces fetal loss and clot formation in mice through complement activation. The CH2-deleted variant (MBB2ΔCH2) of this antibody still binds D1-β_2GPI but fails to activate complement and is not pathogenic [169]. This finding led us to consider the possibility that MBB2ΔCH2 competes with the aβ_2GPI from APS patients, preventing their pathogenic effect. Accordingly, thrombus formation was inhibited when a mixture of MBB2ΔCH2 and β_2GPI-dependent aPL IgG was passively infused in LPS-primed rats. The same molecule administered to pregnant mice significantly reduced fetal death induced by β_2GPI-dependent aPL IgG. In addition, MBB2ΔCH2 was shown to displace patients' aβ_2GPI IgG bound to β_2GPI-coated plates, most likely because of its higher avidity [169]. Altogether these findings support the use of MBB2ΔCH2 as potential new therapeutic strategy for APS miscarriages (Fig. 6.1D).

Group Conclusion

Women with APS are at high risk for recurrent spontaneous miscarriage and late pregnancy complications, such as preeclampsia, IUGR, and preterm birth. These pregnancy complications are a major cause of maternal and fetal morbidity and mortality. Adding further weight to these health issues is the additional problem that it is currently impossible to predict which APS patients will develop an adverse pregnancy event and, if so, which type of pregnancy complication they will suffer. As described here, clinical and experimental observations suggest that the pathophysiology of pregnancy complications in patients with APS may involve complement activation, inflammation, and disruption of normal trophoblast and endothelial function. Nonetheless, there is still much that we do not know, and a better understanding of mechanisms and molecular pathways involved in the pathogenesis of these aPL-associated pregnancy complications will allow us to develop better diagnostics and to identify novel therapeutic targets in order to improve the management and treatment of these patients.

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Part III
Clinical and Diagnostic Aspects of Antiphospholipid Syndrome

Chapter 7
Definition and Epidemiology of Antiphospholipid Syndrome

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Introduction

Thrombotic antiphospholipid syndrome (APS) is characterized by venous, arterial, or small vessel thrombosis with persistent antiphospholipid antibodies (aPL) (lupus anticoagulant [LA] test, anticardiolipin antibody [aCL], and/or anti-β_2-glycoprotein I antibody [aβ_2GPI]). Pregnancy complications such as fetal loss or recurrent early miscarriages are called obstetric APS. A rare variant of APS with multiple intravascular thromboses leading to multi-organ failure is called catastrophic APS (CAPS). Antiphospholipid syndrome can occur as a primary condition (primary APS) or with systemic lupus erythematosus (SLE) or another systemic autoimmune disease. Classification for APS is based on clinical and laboratory criteria (Sapporo APS Classification Criteria), which were established during the 8th International Congress on aPL [1], validated in 2000 [2, 3], and substantially revised in 2006 (Revised Sapporo [Sydney] APS Classification Criteria) [4] (Table 7.1). This chapter describes the broad spectrum of aPL-related clinical problems, epidemiology of APS, and how other diseases mimic APS.

Antiphospholipid Syndrome Based on Revised Sapporo Classification Criteria

The purpose of the Revised Sapporo APS Classification Criteria is to include homogeneous groups of patients in research. The revised version [4], compared to the original [1], includes aβ_2GPI test as one of the laboratory criteria, 12 weeks

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Table 7.1 Revised Sapporo classification criteria for the antiphospholipid syndrome classification criteria [4]

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 histopathologic 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 10th week of gestation
(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia, severe preeclampsia, or recognized features of placental insufficiency
(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory criteria
1. Lupus anticoagulant present in plasma, on two or more occasions at least 12 week apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis
2. Anticardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e., ≥40 GPL or MPL, or greater than the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti-β2-glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titer greater than the 99th percentile) present on two or more occasions, at least 12 weeks apart, measured by a standardized enzyme-linked immunosorbent assay
Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met

instead of 6 weeks interval between two aPL tests, maximum of five years interval between the first aPL and clinical manifestations, and clarified definitions of laboratory thresholds.

While developing the original and the revised criteria, several studies were assessed for the causal role of aPL for clinical manifestations in experimental and clinical studies and the frequency of clinical manifestations in aPL-positive patients. The causal role of aPL was demonstrated in vivo for some (but not all) clinical manifestations [5, 6]. Although several clinical manifestations were frequent in aPL-positive patients [7, 8], suggesting their inclusion as classification criteria, only thrombosis and pregnancy morbidity were eventually selected. Non-criteria manifestations, for instance, livedo, valve disease, and aPL nephropathy, are not included in the criteria because of the lack of recognized causal relationship or statistical association with aPL and also to avoid heterogeneity. Detailed discussion of the limitations of the Revised Sapporo APS Classification Criteria [4] and an international effort to develop new evidence-based APS classification criteria [9] are discussed in Chap. 15.

Clinically Significant Antiphospholipid Antibody Profile

Not every positive aPL test is clinically significant. For instance, transient low titer aPL positivity is common during infections. In fact, more than two-thirds of aPL/aβ_2GPI detected during infections are transient and not associated with clinical consequences [10]; aCL is the most frequent aPL during infections [10]. Thus confirmation of aPL on two occasions at least 12 weeks apart [4] is an important criterion. An isolated aCL/aβ_2GPI test, especially low levels, should trigger an investigation to rule out infection.

The following points are important while correlating aPL tests with clinical events: LA correlates better than do aCL/aβ_2GPI tests [11–14]; moderate to high titer (≥40 U or ≥99th percentile) aCL or aβ_2GPI IgG/IgM (≥99th percentile) is better than are lower titers [15]; IgG isotype is better than IgM isotype [15]; and triple aPL (LA, aCL, and aβ_2GPI) positivity is better than single or double aPL [16, 17]. Clinical judgment is required while interpreting aPL tests when the LA test is performed on anticoagulated patients, especially direct oral anticoagulants, which induce false positive results [18]; aCL or aβ_2GPI IgG/IgM titers are in the lower range (20-40 U); only one aPL determination is available; and/or aCL or aβ_2GPI IgA is the only positive ELISA test.

Antiphospholipid antibody tests that are not part of the current classification criteria, for instance, antiphosphatidylserine-prothrombin or anti-domain I β_2GPI antibodies, may be more specific for APS diagnosis than are "criteria" aPL tests [19]. However, their use in clinical practice is limited due to the lack of standardization (discussed in a different chapter) and limited availability.

Clinical Heterogeneity of Antiphospholipid Antibody-Positive Patients

Antiphospholipid antibodies can result in a broad spectrum of manifestations: criteria APS – thrombotic, obstetrical, or both (Table 7.1), asymptomatic aPL (no thrombosis or pregnancy morbidity); non-criteria manifestations with/without APS classification; and CAPS (multiple-organ thromboses commonly associated with thrombotic microangiopathy).

Several systematic reviews and meta-analyses have been performed on non-criteria manifestations [20, 21]. A recent report summarized recommendations of the APS Clinical Features Task Force of the 14th International Congress on aPL (Rio De Janeiro, Brazil, September 2013); the task force concluded that thrombocytopenia, heart valve disease, renal microangiopathy (aPL nephropathy), chorea, and longitudinal myelitis should be included as part of a future APS Classification Criteria [22]. Table 7.2 summarizes recent meta-analyses assessing selected non-criteria aPL manifestations [20].

"Non-criteria" are not necessarily independent from clinical criteria (Table 7.3). That is, heart valve disease is associated with cerebrovascular events [23], and livedo racemosa predicts stroke [24]. The mechanisms of non-criteria manifesta-

Table 7.2 Selected meta-analysis demonstrating the increased risk of clinical manifestations in antiphospholipid antibody (aPL)-positive systemic lupus erythematosus (SLE) patients compared to aPL-negative SLE patients [20]

Manifestations	Increased risk [OR (95% CI)]			
	LA	aCL	aβ_2GPI	"aPL"
Valve disease	5.8 (2.9–11.8)	5.6 (3.5–8.9)	N/A	3.1 (2.3–4.2)
Pulmonary hypertension	2.0 (1.3–2.9)	2.6 (1.3–5.4)	NS	2.3 (1.7–3.2)
Livedo reticularis	5.7 (3.3–10.1)	3.3 (2.0–5.3)	4.7 (2.4–9.3)	3.6 (2.4–5.4)
Thrombocytopenia	3.9 (2.8–5.4)	1.9 (1.5–2.3)	1.9 (1.0–3.8)	2.5 (2.1–2.9)
Hemolytic anemia	3.7 (2.3–5.9)	2.3 (1.7–3.1)	2.6 (1.2–5.7)	3.0 (2.2–4.2)
Renal impairment[a]	5.3 (2.6–10.9)	4.9 (2.0–12.1)	NS	3.0 (2.0–4.6)

aCL anticardiolipin antibodies, *aβ_2GPI* anti-β_2-glycoprotein-I antibodies, *CI* confidence interval, *LA* lupus anticoagulant, *NS* not significant, *OR* odds ratio

[a]Acute (thrombotic microangiopathy including "glomerular thrombosis" and "intrarenal thrombosis") and/or chronic (for instance, fibrous intimal hyperplasia, focal cortical atrophy) vascular renal lesions

Table 7.3 Statistical associations (*p* value) between antiphospholipid syndrome (APS) clinical criteria and non-criteria manifestations among 600+ patients based on the analysis of the APS Alliance for Clinical Trials and International Networking (ACTION) Registry

MS multiple sclerosis; significant associations are in gray

	Arterial thrombosis	Venous thrombosis	Small vessel thrombosis	Early fetal loss	Late fetal loss	Placental insufficiency
Cardiac valve disease	< 0.001	NS	NS	NS	0.04	NS
Livedo	0.02	NS	0.02	0.01	0.01	NS
aPL-associated nephropathy	NS	NS	< 0.0001	NS	NS	NS
Superficial vein thrombosis	NS	< 0.0001	NS	NS	NS	NS
Skin ulcer	NS	0.001	0.01	NS	NS	NS
Cognitive dysfunction	0.01	NS	NS	NS	0.05	NS
MS-like disease	0.04	NS	NS	< 0.01	NS	NS
Chorea	0.01	0.05	NS	NS	NS	NS
Seizure disorder	< 0.0001	NS	NS	NS	0.046	NS
White matter lesions	< 0.0001	0.0001	NS	NS	NS	NS

tions, for example, endothelial cell activation and vascular wall proliferation, are potentially relevant for criteria manifestations [25, 26]. Antiphospholipid-related nephropathy lesions can be either acute or chronic; acute lesions correspond with microthrombosis (criteria manifestation) [4], whereas chronic lesions correspond with microvascular wall abnormalities such as focal atrophy and intimal hyperplasia (non-criteria manifestation) [27].

Clusters of Antiphospholipid Antibody-Positive Patients

Cluster analysis (CA) is a data-driven method that groups patients so that patients in the same group (cluster) are more similar to each other than to those in other groups. Several studies have used CA to identify different clinical phenotypes in lupus [28], Parkinson's disease, asthma, and inflammatory bowel disease. In APS, CA can characterize the broad spectrum of APS manifestations and to help us understand why patients have heterogeneous presentations.

Based on the APS Alliance for Clinical Trials and International Networking (APS ACTION) clinical database analysis of 497 patients [29], three clusters with different combinations of clinical and laboratory features were identified (Fig. 7.1, Table 7.4):

- Cluster 1 – patients with no other autoimmune diseases, but with venous thromboembolism and triple-aPL positivity
- Cluster 2 – patients with lupus, venous thromboembolism, aPL-related nephropathy, thrombocytopenia, hemolytic anemia, and positive LA test
- Cluster 3 – older patients with arterial thrombosis, heart valve disease, livedo, skin ulcer, neurological manifestations, and cardiovascular (CVD) risk factors

A follow-up analysis of 290 patients with pregnancy history [30] identified four clusters:

- Cluster 1 – older patients with arterial thrombosis and CVD risk factors
- Cluster 2 – patients with pregnancy morbidity only
- Cluster 3 – asymptomatic aPL-positive patients with aCL/aB_2GPI
- Cluster 4 – patients with venous thrombosis obesity, SLE, and LA

These analyses identify clinical phenotypes and suggest that these phenotypes are driven by different triggers or underlying diseases.

Antiphospholipid Antibody (as a Risk Factor) Versus Antiphospholipid Syndrome (as a Disease)

Independent of APS classification criteria, one way of grouping aPL-positive patients is:

- Group 1: aPL is a bystander with no causative role in the clinical problem(s), for instance, a low titer aCL or aβ_2GPI in the setting of an infection.

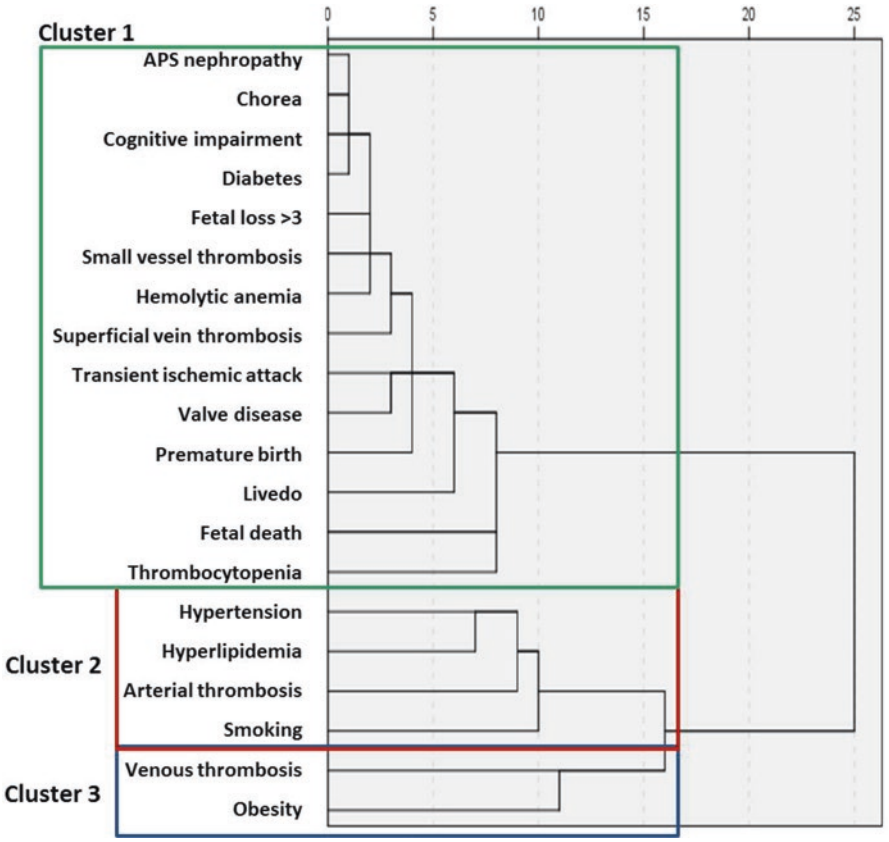

Fig. 7.1 Clustering of the antiphospholipid antibody manifestations based on the analysis of the APS Alliance for Clinical Trials and International Networking (ACTION) Registry. In this analysis, hierarchical clustering was used which encompasses three steps: (**a**) calculating the distance (Euclidean) between means of patients/manifestations aiming to minimize the within-cluster variance; for instance, the distance between two patients with both arterial thrombosis and arterial hypertension is very low (similar patients); however, the distance between a patient with venous thrombosis/obesity and a patient with hemolytic anemia is high (no similarity); (**b**) linking the clusters in a dendrogram (the distance between these clusters is computed using the Euclidean distance, and the two nearest clusters are merged together to form a new cluster that replaces the two previous clusters; merging of the two nearest clusters is repeated until only one cluster is left; the tree diagram generated to illustrate the arrangement of the clusters produced by the clustering is termed a dendrogram); (**c**) comparing clusters of patients

- Group 2: aPL is a risk factor that potentially contributes to the clinical problem(s), for instance, deep vein thrombosis in an aPL-positive patients who is undergoing major surgery.
- Group 3: aPL is the cause of the clinical problems, for instance, non-criteria manifestations of aPL.

Table 7.4 Cluster characteristics of antiphospholipid antibody-positive patients based on the analysis of the APS Alliance for Clinical Trials and International Networking (ACTION) Registry [29]

Variables, *n* (%)	Cluster 1	Cluster 2	Cluster 3	*p* value
Demographics				
Total *n*	179	180	138	
Mean age, year ± SD	41.9 ± 11.6	42.3 ± 12.5	**51.0 ± 12.4**[a,b]	<0.001
Female	**145 (81.0)**[c]	**145 (80.6)**[c]	92 (66.7)	0.004
Past medical history				
Clinical criteria				
Arterial thrombosis	28 (15.6)	51 (28.3)[a]	**95 (68.8)**[a,b]	<0.001
Venous thrombosis	**84 (46.9)**[c]	**85 (47.2)**[c]	45 (32.6)	0.014
Small vessel thrombosis	9 (5.0)	11 (6.1)	10 (7.2)	0.712
Pregnancy morbidity	73 (40.8)	67 (37.2)	42 (30.4)	0.195
Non-criteria manifestations				
Heart valve disease	9 (5.0)	6 (3.3)	**23 (16.7)**[a,b]	<0.001
Livedo	15 (8.4)	26 (14.4)	**30 (21.7)**[a]	0.003
Skin ulcer	6 (3.4)	11 (6.1)	**14 (10.1)**[a]	0.046
Neurological manifestations	22 (12.3)	26 (14.4)	**58 (42.0)**[a,b]	<0.001
aPL nephropathy	2 (1.1)	**10 (5.6)**[c]	0 (0)	0.002
Thrombocytopenia	22 (12.3)	**45 (25.0)**[a]	22 (15.9)	0.006
Other autoimmune diseases				
None	**114 (63.7)**[b]	86 (47.8)	79 (57.2)	0.009
Systemic lupus Erythematosus	25 (14.0)	**74 (41.1)**[a,c]	26 (18.8)	<0.001
Cardiovascular risk factors				
Hypertension on medication	14 (7.8)	33 (18.3)[a]	**99 (71.7)**[a,b]	<0.001
Diabetes on medication	4 (2.2)	5 (2.8)	**12 (8.7)**[a]	0.009
Hyperlipidemia on medication	12 (6.7)	31 (17.2)[a]	**65 (47.1)**[a,b]	<0.001
Obesity	31 (17.3)	49 (27.2)	**60 (43.5)**[a,b]	<0.001
Smoking	44 (24.6)	61 (33.9)	**74 (53.6)**[a,b]	<0.001
Laboratory parameters				
Antiphospholipid antibodies				
Lupus anticoagulant	129 (72.1)	**152 (84.4)**[a]	105 (76.1)	0.017
Anticardiolipin antibodies	**166 (92.7)**[b,c]	63 (35.0)	115 (83.3)[b]	<0.001
Anti-β_2-glycoprotein I antibodies	**138 (77.1)**[b,c]	25 (13.9)	73 (52.9)[b]	<0.001
Triple positivity	**99 (55.3)**[b,c]	13 (7.2)	56 (40.6)[b]	<0.001
Other laboratory parameters				
Hemolytic anemia	2 (1.1)	**18 (10.0)**[a]	6 (4.3)	0.001
Antinuclear antibodies	104 (58.4)	**117 (65.7)**[c]	72 (52.2)	0.05
dsDNA antibodies	43 (24.0)	**61 (33.9)**[c]	23 (16.7)	0.002
Low C3	20 (29.9)	**39 (49.4)**[a]	18 (48.6)	0.039

The variable with the highest percentage, which is significantly more common compared to one other cluster only, is defined as "**Predominant Variable (bold)**" and to two other clusters as "**Discriminant Variable (bold & underlined)**"

[a,b,c]Significantly ($p < 0.05$) more prevalent than Cluster 1, 2, and 3, respectively

Thus, during risk stratification and management decisions, it is important to categorize aPL-positive patients as much as possible, even if at times no clear-cut distinction exists between these groups.

Obstetric Antiphospholipid Syndrome

Recurrent miscarriages, fetal loss, placental insufficiency, or preeclampsia are the criteria manifestations of obstetric APS. They can occur in patients with or without prior thrombosis. The association of pregnancy loss and thrombosis has been described since the first reports of the syndrome [31].

Pregnancy Morbidity Included in the Antiphospholipid Syndrome Classification Criteria [32]

(a) *One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus.*

According to international consensus, fetal death is the most specific criterion for obstetric APS, confirmed by a systematic review [32] and two recent multicenter studies [32, 33]. High titer aCL and aβ_2GPI are associated with a three- to fivefold increased odds of stillbirth [33], while LA positivity is the primary predictor of adverse pregnancy outcome after 12 weeks [34]. Approximately 10–15% of all clinically recognized pregnancies (independent of aPL) result in early miscarriage, most before eight weeks, after which the risk of pregnancy loss decreases significantly (3%). Chromosomal abnormalities, the main cause of spontaneous abortion in the general population, usually result in miscarriage at earlier gestational ages; the incidence decreases steeply after the first trimester, as other causes of fetal loss, such as APS, proportionally increase [35]. Congenital abnormalities, regardless of karyotype, play a significant role in all fetal loss. Obstetric APS diagnosis and classification exclude these alternative causes of fetal loss.

(b) *One or more premature births of a morphologically normal neonate before the 34th week of gestation due to (i) eclampsia or severe preeclampsia defined according to standard definitions or (ii) recognized features of placental insufficiency.*

Preeclampsia is relatively common in a general population (5–10% of all pregnancies) [36]. In APS patients, the incidence of preeclampsia is higher; half of the cases are classified as severe. The real frequency of aPL in patients with preeclampsia is still unknown, but many studies report a significant association between aPL

and early-onset severe preeclampsia [37]. Recently, a meta-analysis of 12 studies and 8475 SLE patients showed an odds ratio of 2.86 (95% confidence interval [CI]: 1.37–5.98) for preeclampsia of any severity in SLE patients with aPL, compared to those without aPL [38].

Preeclampsia has a standard definition: systolic blood pressure ≥140 mmHg or diastolic ≥90 mmHg on two occasions, at least four hours apart at 20 or more weeks, and ≥300 mg protein/24-h urine collection in a previously normotensive patient. Severe preeclampsia is when the systolic blood pressure is ≥160 mmHg or diastolic ≥110 mmHg on two occasions, at least four hours apart, and/or HELLP syndrome (see below), renal insufficiency, pulmonary edema, and new-onset cerebral or visual disturbances such as headache or blurred vision occur. The occurrence of generalized tonic-clonic seizures defines eclampsia [39].

Intrauterine growth restriction due to placental insufficiency in APS patients is high (15–40%), although the definition of placental insufficiency is debatable [40, 41]. The Revised APS Classification Criteria describe placental insufficiency as: abnormal or non-reassuring fetal surveillance test(s), for instance, a nonreactive non-stress test, suggestive of fetal hypoxemia; abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, for instance, absent end-diastolic flow in the umbilical artery; oligohydramnios, for instance, an amniotic fluid index of five centimeters or less; and a postnatal birth weight less than the 10th percentile for the gestational age [4]. The differential diagnosis of placental insufficiency includes SLE, multiple gestation, substance abuse (tobacco, alcohol, or cocaine), infections, genetic and structural disorders, and placental abnormalities [40].

To enhance the specificity of this criterion, the Revised APS Classification Criteria include only cases that are complicated by preterm delivery before 34 weeks. This criterion may be insensitive and nonspecific. Delivery is usually due to the intervention for fetal or maternal reasons; spontaneous preterm labor of an otherwise uncomplicated pregnancy would be unusual in APS [37].

(c) *Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and/or paternal and maternal chromosomal causes excluded.*

Recurrent early abortion before the 10th week of gestation is the most sensitive obstetric criterion [4], although not specific. The main limitation of this definition is the difficulty to exclude other causes. Underlying problems such as uterine abnormalities can be easily diagnosed, but embryonic chromosomal abnormalities are usually not investigated in clinical practice. Abnormal embryonic karyotype is the most common cause of recurrent miscarriage and of single spontaneous abortion [42, 43].

Inability to exclude other causes of recurrent early miscarriages, combined with different inclusion criteria, may explain the conflicting results in observational studies and clinical trials [44]. Because well-designed studies of patients with clinically significant aPL profiles are limited, some authors question the association between recurrent early miscarriage and aPL [45].

Other Potential Antiphospholipid Antibody-Related Pregnancy Morbidity

HELLP syndrome is an acronym for *h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelet count. It usually occurs as a severe presentation of preeclampsia; in 15–20% of cases, it develops without hypertension or proteinuria [46]. Differentiating HELLP syndrome from preeclampsia can be difficult, as overlapping clinical findings such as hemolysis, platelet consumption, renal failure, and thrombotic microangiopathy characterize both situations [47]. If an aPL-positive patient develops HELLP syndrome, the clinical course is severe, for instance, early onset and liver infarcts; after delivery, maternal health may be compromised by irreversible liver or kidney damage [48].

There is also an ongoing debate whether the definition of obstetric APS criteria should be broadened, especially considering the number of early miscarriages and low aPL titers. The literature is conflicting, and well-designed studies are lacking. There is limited consensus; some physicians manage patients as if they have APS even if they do not fulfill the Revised Sapporo APS Classification Criteria [44]. Mekinian et al. described a high rate of obstetric events in patients with low aPL titers, similar to those of patients with moderate-to-high titers [49]. On the contrary, Simchen et al. described a normal proportion of successful pregnancy outcomes (77%) in patients with low titers (35% of all patients) [50]. Based on randomized trials, there is no evidence that patients with positive aPL and two early pregnancy losses (before 10 weeks of gestation) should be treated [51]. Also, the difficulty of excluding chromosomal abnormalities as a cause of miscarriage may lead to incorrect interpretations of the results; treatment with heparin and low-dose aspirin for previous abortions due to aneuploidies will not improve outcomes [51]. To provide evidence-based data in these controversial questions, the members of the Obstetric Task Force of the 14th International Congress on aPL agreed that trials should be considered in patients with low positive aPL titers and also in high titer aPL patients with one or two early miscarriages [44] (discussed in Chap. 12).

What Is Not Obstetric Antiphospholipid Syndrome?

Infertility is a common condition and has been tentatively linked to aPL for decades. In vitro studies have demonstrated that aPL can disrupt the anticoagulant annexin A5 shield on trophoblast, induce a pro-inflammatory response, and reduce trophoblast proliferation and invasion. Thus, in theory, aPL could interfere with early stages of uterine decidualization and result in infertility [52]. However, clinical studies have failed to prove an association. Although some studies identified increased frequency of aPL in infertility patients (compared to controls), the results are arguable, considering the heterogeneity of the clinical, laboratory, and methodological aspects of these studies, for instance, the

definition of aPL positivity and small number of participants. Also, several studies included non-criteria aPL tests that have controversial clinical significance [53]. A recent review reported no association between aPL and assisted reproductive therapy (ART) achievement of pregnancy and no benefit of treatment for aPL [52]. These conclusions have been supported by the American Society of Reproductive Medicine [54].

Placental abruption is not part of the Revised Sapporo APS Classification Criteria, and no pathological mechanism exists to describe a connection. Hypertensive disorders are identified in half of patients with placental abruption [55], a significant confounding factor. Observational studies demonstrate an increased incidence of placental abruption in aPL-positive patients; however, hypertension, which is common in APS, is a significant confounding factor.

Microangiopathic and Catastrophic Antiphospholipid Syndrome

Microangiopathic aPL-associated syndrome (MAPS) and CAPS are rare variants of APS that clinically present as "thrombotic storm" [56], an acute development of multiple thromboembolic events involving diverse vascular beds.

Microangiopathic Antiphospholipid Antibody-Associated Syndrome

Microthrombosis (kidney, heart, liver, lung, and skin) can occur in aPL-positive patients in single or multiple organs. The term "microangiopathic aPL-associated syndrome" was originally proposed by Asherson et al. [57–61] to describe several conditions with endothelial dysfunction [62, 63], which may occur with aPL (Table 7.5). By the term *associated*, the authors emphasized that many of these conditions may occur in patients who do not have aPL; they also hypothesized that aPL appears in response to exposure of phospholipids as cells suffer damage, a concept not accepted today. The importance of this concept for clinicians lies in the fact that in these conditions, even when aPL is positive, therapy may differ from that directed at CAPS [61].

Table 7.5 Selected diseases with thrombotic microangiopathies (refer to Chap. 17 for differential diagnosis)

1. Catastrophic antiphospholipid syndrome
2. Thrombotic thrombocytopenic purpura
3. Hemolytic uremic syndrome
4. Disseminated intravascular coagulation
5. HELLP syndrome

Catastrophic Antiphospholipid Syndrome (CAPS)

Catastrophic APS is a rare variant of APS presenting with multiple intravascular thromboses, usually affecting small vessels throughout the body, and leading to multi-organ failure. Catastrophic APS occurs in less than 1% of APS patients but has a high mortality rate [62].

The classification criteria for CAPS were developed at a preconference workshop during the 10th International Congress on aPL held in Taormina, Italy, in 2002 [63]. The criteria include (a) involvement of three or more organs, systems, and/or tissues, (b) development of manifestations simultaneously or in less than a week, (c) histopathological confirmation of microvascular thrombosis, and (d) positive aPL. The classification is definite CAPS if four criteria are satisfied; if only three criteria are satisfied, it is called probable CAPS. In clinical practice, challenging patients may not fulfill the definite or probable CAPS criteria and are called CAPS-like; they require close monitoring for CAPS development and may require aggressive management similar to CAPS [64]. Antiphospholipid antibody-positive patients with medium- to large-vessel thromboses in two organs with or without concurrent bleeding, isolated microthrombosis with bleeding (pulmonary or adrenal hemorrhage), severe thrombocytopenia with or without bleeding, and severe HELLP syndrome with single organ thrombosis are included in this CAPS-like group [64].

Previous APS diagnosis and/or persistent clinically significant aPL positivity is of great importance for the CAPS diagnosis; however, almost half of patients who develop CAPS do not have a history of aPL positivity. Thus, at times CAPS diagnosis can be challenging. Diagnostic algorithms for CAPS providing a "step-by-step" approach for clinicians in the assessment of patients with multiple-organ thromboses are available [64].

An international CAPS registry compiles cases with this condition; three descriptive studies have been published [65–67]. Infections, drugs, major/minor surgical procedures, and anticoagulation withdrawal are some of the precipitating factors reported for CAPS. A majority of patients show thrombotic microangiopathy features. Detailed discussion of CAPS diagnosis, differential diagnosis, and management can be found in a different chapter.

Epidemiology of Antiphospholipid Syndrome

The number of epidemiological studies on APS is limited. Only a few large APS cohorts are available to estimate the distribution of APS in different genders, races, and geographic regions [68, 69].

Prevalence of Antiphospholipid Antibodies in General Population

Although the prevalence of aPL (low or high titer) approaches is 10% in a general healthy population, persistent LA or moderate-to-high titer aCL/aβ_2GPI positivity is uncommon. A prospective study of healthy blood donors who were tested twice for aPL demonstrated, at baseline, 10% and 1% positivity for aCL and LA, respectively; after 1 year, fewer than 1% tested positive for either test [70]. In healthy pregnant women at 15–18 weeks of gestation, the prevalence of aβ_2GPI and aCL is 3.9% and 1.6%, respectively [70]. In another study, the prevalence of aCL or LA in 500 pregnant women was 3% [71].

Based on a literature review of 120 full-text papers, aPL frequency was estimated as 6% for patients with pregnancy morbidity, 13.5% for stroke, 11% for myocardial infarctions, and 9.5% for deep vein thrombosis. Limitations of the literature are that 60% of the papers were published before 2000, all three criteria aPL tests were performed in only 11% of the papers, 36% of papers used a low-titer aCL cutoff, aβ_2GPI cutoff was heterogeneous, aPL confirmation was performed in only one-fifth of papers, and the study design was retrospective in nearly half of the papers. The authors concluded that it is difficult to determine the frequency of a clinically significant aPL profile in patients with aPL-related clinical outcomes due to the lack of robust data [72].

Another literature review of 43 studies of patients with cardiovascular events included 5217 subjects (patients and controls) [73]. Antiphospholipid antibody prevalence for any cardiovascular event was 17% (range 5–56%), for stroke 17% (2–56%), and for TIA 12% (2–45%). Overall (1081 patients and 1868 controls), 13 out of 15 studies (87%) reported significant associations between aPL and cardiovascular events, with a cumulative OR of 5.5 (95% CI: 4.42 to 6.79). The frequency of aPL in young (<50 years old) patients with cardiovascular events was estimated at 17% for all events and antibody types and 22% for aCL in patients with stroke. An important methodological limitation in this study is variability in test reproducibility and cutoff definitions [73].

Prevalence of Antiphospholipid Antibodies in Systemic Autoimmune Diseases

In SLE, 30–40% of patients are positive for aPL; when each aPL is investigated individually, the prevalence of a positive LA test and aCL varies between 11–30% and 17–40%, respectively. In a cohort of 262 SLE patients, clinically significant aPL profile (described above) was 33% [74]. Mok et al. reported a prevalence of LA, aCL, and aβ_2GPI as 22%, 29%, and 8%, respectively in Chinese SLE patients

[75]. Another review reported aPL prevalence in SLE patients in different geographical regions as 18–27% in North America, 16–39% in South America, 14–31% in Europe, 13–17% in Africa, and 13–44% in Asia [76].

Gender of Antiphospholipid Syndrome Patients

Lupus has a 9/1 female/male ratio; the majority of patients are 15–50 years old [68]. In a European cohort of 1000 APS patients (Europhospholipid Project), the female/male ratio was 5.0, being 7.0 in patients with SLE and 3.5 in patients without SLE [68]. After excluding obstetric APS, the female/male ratio was 1.0 in patients without SLE. A specific analysis of the APS Alliance for Clinical Trials and International Working (APS ACTION) Clinical Database (638 patients) for this chapter showed female/male ratios of 4.2, 2.7, and 1.4 in APS patients with SLE, without SLE, and without SLE and obstetric morbidity, respectively.

Clinical presentations of male and female APS patients differ: in a cross-sectional study on primary APS patients, females had higher frequency of pulmonary embolism and IgM aCL [77]; another study showed female predominance for cerebrovascular events and a male predominance for gastrointestinal thrombosis [78]. In neither study there was a difference between two groups with respect to overall venous and arterial thrombosis.

Racial Distribution of Antiphospholipid Syndrome Patients

The racial distribution of APS is unknown. Early studies investigating prevalence of aPL (for any aCL isotype) in primary or SLE-related APS reported 44–88% of patients as Europeans, 11–33% African-Americans, 7–53% Hispanics, and 17–46% Asians [79]. The wide range is likely due to the heterogeneous patient groups tested with no standardized aPL tests. The significance of the low frequency of IgG aCL in Afro-Caribbeans and the possible correlation to the aPL-related events are not clear. Diri et al. [80] reported a case series of eight African-American APS patients that stated that IgA is the most frequent isotype of aCL and aβ_2GPI; IgM isotype accompanied IgA in three of the four patients with neurologic manifestations.

Age of Onset in Antiphospholipid Syndrome

Based on the Europhospholipid Project, the prevalence of aPL increases with age; up to 50% of elderly patients with chronic disease were reported to have positive aPL. Patients with older onset APS were predominantly male and had higher incidence of arterial thrombosis [68].

What Mimics Antiphospholipid Syndrome?

Antiphospholipid syndrome should be included in the differential diagnosis of thrombosis (especially young patients with no other risk factors) and relevant obstetric morbidity; however thrombosis is usually multifactorial, and at least half of APS patients with thrombosis have additional risk factors at the time of their events [81, 82]. In selected conditions, given aPL positivity (clinically significant or not), physicians may not have a broad differential diagnosis. Microangiopathic aPL-associated syndrome was discussed above. Infections (particularly leprosy), Behcet's disease, and malignancy-associated thrombosis are described below, given the similarity of signs and symptoms with APS [83].

Infections

Although infections generally result in transient aPL (usually IgM isotype) [84, 85], some trigger both aPL and clinical manifestations resembling those of APS [83, 85]. Table 7.6 shows common infections associated with aPL. As discussed above, an isolated aCL/aβ_2GPI test, especially low levels, should trigger an investigation to rule out infection.

Table 7.6 Infectious diseases associated with positive antiphospholipid antibodies (aPL) with/without aPL-related clinical symptoms [10]

1. Viral	
Hepatitis C virus	Varicella
Epstein-Barr Virus	Vaccinia
Human immunodeficiency virus	Mumps
Cytomegalovirus	Rubella
Parvovirus B19	Human T-cell lymphotropic virus-1
Adenovirus	
2. Bacterial	
Leprosy	Staphylococci
Tuberculosis	Streptococci
M pneumoniae, *M. penetrans*	*Coxiella burnetii* (Q fever)
Salmonella	Bacterial endocarditis
3. Spirochetal	
Syphilis	Lyme disease (*Borrelia burgdorferi*)
Leptospirosis	Pinta (*Treponema pallidum carateum*) Rat-bite fever (*Spirillum minus*)
4. Parasitic	
Malaria	Toxoplasmosis
Kala azar	

Leprosy is an infectious disease caused by *Mycobacterium leprae* that mostly involves the skin and peripheral nervous system, but can also present with fever, arthritis, pericarditis, and glomerulonephritis. Definitive diagnosis is established based on full-depth skin or nerve biopsy smears demonstrating acid-fast positive bacilli (AFB) stain for lepra bacilli [86]. Physicians should consider leprosy in patients from endemic areas, as differential diagnosis for SLE and APS, as leprosy patients develop antinuclear antibodies, anti-dsDNA, anti-mitochondrial antibodies, and aPL [86, 87].

The reported prevalence of aPL in leprosy has a wide range (aCL 20–98%; $a\beta_2$GPI 3–89% of patients) [88–90], possibly explained by the fact that infection-induced aPL are usually transient. However some leprosy patients develop persistent, autoimmune aPL [91]. Although IgM is the most common aCL isotype, IgG also occurs, mostly in the lepromatous form [88].

Leprosy patients may develop a wide spectrum of clinical and laboratory manifestations [86]. Type 1, seen mostly in tuberculoid leprosy, is a mild clinical presentation mostly with IgM aCL; it represents a slight increase in cell-mediated immunity. Type 2, mostly in lepromatous leprosy, is a severe clinical presentation mostly with IgG aCL; it causes an antigen-antibody complex-mediated immune complex disease with complement activation and the morphologic expression is leukocytoclastic vasculitis, with expression of tumor necrosis factor and interferon-γ.

Lucio's phenomenon is a rare necrotizing skin lesion of leprosy in which patients develop small vessel thrombosis similar to that seen in APS patients. Biopsies show microthrombosis without inflammation. The abundant bacilli are thought to cause microthrombosis by endothelial proliferation and occlusion [92].

Behçet's Disease

Behçet's disease (BD) is a chronic, multisystem inflammatory vasculitis characterized by mucocutaneous, ocular, vascular, arthritic, and neurological involvement. It is also characterized by recurrent vascular thrombosis and vasculitis. The cause and pathogenesis are unclear, but various immunological abnormalities associated with both humoral and cellular immune systems have been reported. The diagnosis is mostly clinical [93]. Although previous studies suggest an increased frequency of aCL in BD, the low numbers of patients in most of these studies, especially patients with thrombotic complications, make it difficult to draw conclusions [94, 95].

Tokay et al. assessed the point prevalence and clinical relevance of aCL in BD in 128 patients: the point prevalence was 7% [96]. Alekberova et al. reported in their cohort 20% of BD patients were positive for aPL, either aCL (median IgG and IgM 33 GPL and 41 MPL, respectively) or LA. Further analysis did not link thrombotic complications with aPL [94].

Hughes-Stovin syndrome (HSS) is a rare disorder of pulmonary artery aneurysm in the setting of systemic thrombosis [97]. The term "incomplete Behçet's Disease" has been used to describe this syndrome. Although patients with HSS lack typical BD presentation, given the clinical, radiological, and histopathological similarities between HSS and BD, HSS may be a variant of BD [98, 99] or, in fact, may be BD [100].

Malignancy

Armand Trousseau noted an association between hypercoagulability and cancer in 1865 [101]. Trousseau's syndrome describes recurrent episodes of venous and/or arterial thrombosis occurring in patients with underlying malignancy [102]. Deep vein thrombosis and pulmonary embolism occur in 10–50% of patients with cancer [103]. Thrombosis is associated with all kinds of cancers and is triggered by tissue factor, cancer procoagulant, and/or various cytokines [103]. Livedo reticularis can also appear as a clinical manifestation of cancer, especially lymphoproliferative malignancies through hyperviscosity, hyperproteinemia, or thrombosis [104].

The association of aPL and cancer has been under investigation for several years. Small studies in Caucasian populations have shown that up to one-third of cancer patients test positive to aPL [104, 105]. Yoon et al. analyzed 33 cancer patients with thrombosis and showed that aPL (LA, aCL, and/or aβ_2GPI) was positive in 61% [106]; aCL and aβ_2GPI positivity were defined as more than 20 standard units. Given the large discrepancy in the literature about the frequency of aPL, there is no evidence to support the screening of aPL-positive patients for a malignancy or screening the malignancy patients for aPL.

Group Conclusion

Although aPL increases the risk of thrombosis and pregnancy morbidity, APS as a disease with broad spectrum of clinical manifestations needs to be better defined. Clustering APS patients according to different clinical manifestations and risk stratification will help physicians and researchers understand the disease characteristics better. Although there are many speculations, epidemiology of APS is yet to be elucidated by large prospective cohort studies with different age groups, geographical areas, and races.

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Chapter 8
Clinical and Prognostic Significance of Non-criteria Antiphospholipid Antibody Tests

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Introduction

Classification criteria for antiphospholipid syndrome (APS) require IgG and IgM isotypes of the anticardiolipin antibodies (aCL), anti-β_2-glycoprotein I antibodies (aβ_2GPI), and/or the lupus anticoagulant (LA) to satisfy the laboratory criterion for disease definition [1]. However, over the past 20 years, several other "non-criteria" antiphospholipid antibodies (aPL), directed to other proteins of the coagulation cascade (i.e., prothrombin and/or phosphatidylserine–prothrombin complex), to some domains of β_2GPI, or that interfere with the anticoagulant activity of annexin A5, have been proposed [2]. In some cases, these assays detect specific subsets of pathogenic antibodies or a particular mechanism in APS. The Laboratory Diagnostics Task Force at the 14th International Congress on aPL (Rio de Janeiro, Brazil, 2013) highlighted several non-criteria assays [3]. However, there was consensus that further studies are necessary to obtain high-quality evidence defining their overall roles as risk predictors.

The task force reviewed the literature and conducted new studies between 2013 and 2016; the conclusions were presented at a special session during the 15th International Congress on aPL (www.apsistanbul2016.org, North Cyprus, September 2016). This paper updates our recommendations.

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Phosphatidylserine-Dependent Antiprothrombin Antibodies

Many reports show the clinical utility of phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) assay in the diagnosis of APS, a conclusion of a task force at the 13th International Congress on aPL (Galveston, TX, 2010) [4] and reviewed, in an evidence-based manner, during the 14th International Congress on aPL (Rio de Janeiro, Brazil, 2013) [3]. The inclusion of aPS/PT antibodies as a laboratory criterion of APS was considered unwarranted then because of poor standardization of its assay and because reproducibility of the strong correlations between aPS/PT and APS manifestations needed confirmation in larger studies.

A recent systematic review suggests that aPS/PT does represent a strong risk factor for arterial and/or venous thrombosis [5]. A group of scientists led by Amengual and Atsumi carried out initial and validation retrospective cross-sectional multicenter studies on aPS/PT [6]. The initial retrospective study acquired data from eight centers from seven countries. Serum/plasma samples were blindly tested for IgG aPS/PT at Inova Diagnostics Inc., United States (USA), using enzyme-linked immunosorbent assay (ELISA) kits provided by two manufacturers: the QUANTA Lite™ aPS/PT IgG ELISA, a Food and Drug Administration (FDA)-approved assay from Inova, and the PS/PT ELISA kit for IgG isotype from Medical and Biological Laboratories Co. Ltd., Nagano, Japan. After completing the initial study, a validation study, using the same methodology for a new cohort of samples from five countries, was carried out.

The initial study comprised 247 subjects. A correlation was obtained with both ELISA kits for the IgG aPS/PT ($r = 0.827$, $p < 0.001$). Two hundred and four samples with concordant IgG aPS/PT results in both ELISAs were subsequently analyzed (99 APS, 58 non-APS, and 47 healthy). Immunoglobulin G aPS/PT were more prevalent in APS patients (51%) than in those without (9%), with an OR of 10.8 [95%CI 4.0–29.3], $p < 0.0001$. For APS diagnosis, sensitivity, specificity, and positive (LR+), and negative likelihood ratio (LR-) were 51, 91, 5.9, and 0.5%, respectively. In the validation study ($n = 214$), a significant correlation was found for IgG aPS/PT titers ($r = 0.803$, $p < 0.001$). Immunoglobulin G aPS/PT concordant samples were again analyzed ($n = 182$; 76 APS, 57 non-APS, and 49 healthy). Immunoglobulin G aPS/PT were more frequently found in APS patients (47%) than in those without (12%), with an OR of 6.4 [95%CI 2.6–16], $p < 0.0001$. For APS diagnosis, sensitivity, specificity, and LR+, and LR- were 47%, 88%, 3.9%, and 0.6%, respectively.

Whether to include non-criteria antibodies in the designation aPL-positive is still under discussion. Current APS classification criteria exclude patients with clinical manifestations suggestive of APS who have non-criteria antibodies, sometimes referred to as seronegative APS [7]. The above multicenter study confirms, in both cohorts, high prevalence of IgG aPS/PT in patients with definite APS and in those with APS-associated clinical manifestations in the absence of APS laboratory criteria [6]. Thus, based on the available evidence, the task force suggests the inclusion of IgG aPS/PT in the APS classification criteria.

Antibodies to Domains of β_2-Glycoprotein I

β_2-Glycoprotein I (β_2GPI) is the main antigenic target for aPL [8]. Anti-β_2GPI may activate the endothelial cells, monocytes, and platelets, triggering the coagulation cascade by recognizing membrane- or receptor-bound β_2GPI [8–10]. Dimerization of β_2GPI or the complexing of β_2GPI with antibodies stabilizes receptor affinity, allowing cell signaling to occur [11].

Experiments in mouse models of APS show that patient-derived autoantibodies against β_2GPI increase the thrombotic risk. Remarkably, epidemiologic studies do not show a strong relation between these antibodies and thrombosis or pregnancy morbidity. Compared to the LA test, the correlation is weak. There are a number of explanations for this incongruity. Lack of standardization of the ELISA results in large differences in results obtained in sample exchange programs. Another possibility is that the ELISAs pick up irrelevant low-affinity antibodies, which lead to many positive results (false-positives) in healthy individuals. A third possibility is that the aβ_2GPI ELISA measures a heterogeneous population of antibodies, and not all autoantibodies directed against β_2GPI are a risk factor for thrombosis or fetal loss.

Antibodies to Domain I of β_2-Glycoprotein I

Many groups have used isolated domains or peptides to study the specificity of autoantibodies against β_2GPI [12–16], concluding that antibodies directed against a specific peptide sequence in domain I (DI) of β_2GPI (Arg39-Arg43) have higher correlation with thrombosis than do antibodies directed against the whole molecule; antibodies directed against other domains of β_2GPI do not. Reactivity against DI is associated with clinical APS and with LA, suggesting a higher diagnostic/prognostic value for anti-DI β_2GPI [17].

These correlations found in patient populations are confirmed in animal models of APS. A human monoclonal aβ_2GPI reacting with both the peptide and the whole DI is pathogenic in animal models [18]. When mice are injected with patient antibodies enriched with DI-specific antibodies, the mice become prothrombotic. When mice are injected with patient antibodies free of DI-specific antibodies, no prothrombotic phenotype is observed [19]. Other studies show that addition of purified DI to aPL completely attenuates the prothrombotic effects of these antibodies in mice. When amino acid arginine 39 of DI is replaced by serine, the anti-thrombotic effect of DI disappears [20]. These experiments show that autoantibodies against DI of β_2GPI are pathologic. Whether this is the only pathologic antibody population is uncertain.

Pelkmans et al. isolated human B-cell monoclonal antibodies against DI of β_2GPI [21]. Characterization of two of these antibodies shows that they do not mutually compete for binding to β_2GPI, indicating that they recognize different epitopes. Indeed, one of the antibodies, P1–117, recognizes the domain around amino acids Arg39-Arg43, while the other, P2–6, recognizes another part of DI. These two human

monoclonal antibodies have been used to validate commercial assays that detect autoantibodies against β_2GPI. The epitope recognized by the autoantibodies directed against epitope Arg39-Arg43 is cryptic in the form in which β_2GPI circulates in plasma; hence these antibodies do not recognize circulating β_2GPI. After binding to anionic phospholipids or other negatively charged surfaces, β_2GPI undergoes a conformational change with results in the exposure of epitope Arg39-Arg43 [22].

A prerequisite for a good ELISA to detect autoantibodies against β_2GPI is optimal coating of β_2GPI. Improper coating will result in (partly) shielding of the important epitope in β_2GPI. Testing different commercial ELISAs with the two human monoclonal antibodies showed that some commercial ELISAs recognize P2–6 much better than P1–117, indicating that in these ELISAs, β_2GPI was incompletely unfolded. Indeed, a study in a larger patient cohort showed that these commercial ELISAs could not detect the low titer autoantibodies against DI of β_2GPI. Apparently, at least part of the variability with different commercial assays can be explained by incomplete unfolding of β_2GPI.

Some precautions should be taken when attempting to measure DI autoantibodies in an ELISA in which DI is directly coated. The epitope to which the autoantibodies are directed is positively charged. Using a hydrophilic ELISA tray will result in binding of this epitope to the positive charge of the tray, resulting in shielding of this epitope from the antibodies; it is essential to use a hydrophobic ELISA tray [17].

Some other points are debated. For example, the facts that anti-DI antibodies can be detected more frequently than anti-DIV-V antibodies in patients with double- or triple-positive aPL classification tests, and are more strongly associated with LA positivity, raise the issue whether the predictive power is dependent on this antibody subpopulation or is simply linked to a high-risk aPL profile. Moreover, despite higher specificity, anti-DI assay apparently has lower sensitivity in comparison to the assay with the whole molecule [23].

The task force concluded that it is too soon to recommend replacement of aβ_2GPI testing by anti-DI testing.

Antibodies to Domain IV-V of β_2-Glycoprotein I

While the use of domain-deletion mutants shows that the immunodominant epitope resides in DI [12], antibodies against peptides of different domains have been described [15].

Antibodies against DIV-V show lower specificity for APS or systemic autoimmune conditions than do anti-DI antibodies. Antibodies against DIV-V are more frequent in asymptomatic aPL carriers, in patients with leprosy, in children suffering from atopic dermatitis, and in children born from mothers affected by systemic autoimmune disorders [23, 24]. Anti-DI antibodies may cluster in patients with autoimmune diseases; the ratio of antibodies targeting DI to those targeting DIV-V may discriminate among antibodies more linked to the syndrome [23]. Anti-DI, but not anti-DIV-V, antibodies occur in obstetric APS patients, even in patients without vascular events [23, 25].

The task force concluded that antibodies against DIV-V of β_2GPI show lower specificity for APS or systemic autoimmune conditions than do anti-DI antibodies. Thus, due to the unavailability of the assay to detect these antibodies, no recommendations are given on this subject.

Immunoglobulin A Anticardiolipin and Anti-β_2Glycoprotein I Antibodies

Immunoglobulin G and IgM aCL were first accepted as valid measures in the 1980s; IgA was not accepted because of high variability among laboratories. When consistent measurement was assured, it became apparent that, rarely, IgA aCL might be the only detectable antibody [26] and that IgA aCL occurs in up to 40% of patients with SLE [27–29]. Recent studies in SLE report a prevalence of 16 to 58% for IgA aβ_2GPI, particularly among those of African-American ethnicity [27–29], and in patients with the primary APS up to 72% [30–34]. In 1995, Pierangeli showed that IgG, IgM, and IgA aCL are pathogenic in a mouse thrombosis model [35]. Later, she showed that aβ_2GPI isolated from four APS patients with only IgA upregulated tissue factor and caused thrombosis in mice [36].

It is not yet clear if measurement of IgA aPL is useful for everyday practice. Some authors emphasize that methodological problems and lack of standardization still exist among commercial preparations and that the addition of IgA to IgG and IgM does not identify increased thrombosis risk in SLE patients.

Tincani et al. used a homemade ELISA for IgA aβ_2GPI to demonstrate positive tests in 28%, 40%, and 3% of 119 APS, 328 SLE, and 78 healthy controls ($p < 0.0001$ for both patient groups). In SLE patients positive IgA and IgG isotype prevalence was similar, while IgM was lower; in primary, APS IgG and IgM were the most frequent isotypes. Among patients with primary thrombotic APS, 65% of the 31 subjects with recurrent thrombotic episodes had aβ_2GPI IgA compared to 39% of the 46 with one episode ($p < 0.05$) [37].

The reported experience shows that the routine performance of IgA aβ_2GPI may be useful in SLE patients, as reported by others [38] but also in primary APS, where these antibodies might have a prognostic value. Furthermore, other authors suggested that IgA aβ_2GPI can be an independent risk factor for the development of the first aPL-related event, particularly arterial thrombosis [39].

The task force suggests appropriate prospective studies that will allow the evaluation of IgA antibody as a thrombosis risk factor are still needed.

APhL Assay

The APhL assay uses a mixture of negatively charged PL antigens, phosphatidylserine (PS) and phosphatidic acid (PA), with β_2GPI (Louisville APL Diagnostics). It has high sensitivity for identifying APS patients with typical clinical manifestations and

has improved specificity when disorders other than APS (e.g., infectious and autoimmune diseases), which often give false-positive aCL results, are studied [40]. The assay derives from older experiments (that antedate discovery of β_2GPI) demonstrating that serum from infectious disease patients and that from autoimmune disease patients differ in their binding to various phospholipids [41]. Sera from syphilis patients had very low affinity for PS and PA despite a high affinity for cardiolipin (CL), while sera from autoimmune patients had high affinity for CL, PS, and PA. Identification of a mixture of two negatively charged phospholipids that enabled the best distinction resulted in the creation the APhL assay in the 1990s [40]. Over 20 years, this test has been proven to identify nearly all aCL-positive APS patients (sensitive) and is usually negative for patients with infectious and other autoimmune diseases (specific) [42–46]. The original assay did not consider the role of β_2GPI; whether it performs the same in the presence and absence of β_2GPI is unknown.

An independent study from Suh-Lailam et al. [47] showed comparable sensitivity for APS between the APhL assay and the aCL assay, when infection-induced antibody was defined by populations of patients with syphilis and parvovirus B19; the specificity of the APhL was greater. The APhL assay also compared favorably with the aβ_2GPI assay in this study, with a sensitivity of 88% and specificity of 98%. (A weakness of this study is that only 16 of 101 aPL-positive patients had known APS; the remainder were drawn from samples submitted to a commercial laboratory and found to be positive.) A review in 2000, which included sera from patients with APS, leishmaniasis, leptospirosis, and syphilis, stated that the aCL assay was positive in 100% of APS sera, the APhL in 98%, and the aβ_2GPI in 74%. Specificities for identifying APS were 73% for aCL, 96% for APhL, and 70% for aβ_2GPI. The aCL and aβ_2GPI tests were more frequently positive in infectious disease sera, making them less specific than the APhL test [48]. In data presented in this study, details of the sources of infectious disease sera are not provided; specificity was calculated using 42 non-APS samples, stated to derive from patients with syphilis, human immunodeficiency virus, Q fever, and other non-APS autoimmune diseases.

These studies suggest that the APhL assay might serve as an alternative to aCL as a first-line test in the APS diagnostic algorithm. Results from a wet workshop at the 13th International Congress on aPL that evaluated the performance of aCL, aβ_2GPI, and APhL assays in the identification of 26 APS and persistent aPL-positive patients versus 21 healthy, infectious disease, and autoimmune controls supports this assertion [49]. The report from the 14th International Congress on aPL called for more extensive testing to confirm this assertion, especially for autoimmune diseases for which data are lacking [3]. Consequently, a comparative analysis was performed in a large number (*n*: 1178) of well-characterized SLE patients from ethnically diverse SLE cohorts [50]. In this study, IgG aβ_2GPI were highly associated with venous thrombosis in SLE patients, while the APhL and aCL assays were also associated with venous thrombosis, but with smaller OR values. The APhL was the only assay associated with both venous and arterial thrombotic manifestations.

A critical review of the APhL assay was presented at the 15th International Congress on aPL [51]. Six articles met selection criteria; in all the APhL assay correlated with APS, and OR values were greater than those for aCL and aβ_2GPI. The

specificity of the APhL assay in diagnosing APS was greater than that for the aCL assay but similar to aβ_2GPI in most studies. Conversely, the APhL assay showed similar sensitivity for APS diagnosis when compared to the aCL assay and improved sensitivity when compared to the aβ_2GPI assays.

The task force concluded that more data on the clinical utility of APHL test are needed before any recommendation can be reached.

Antibodies to Factor Xa

Numerous studies show interactions of monoclonal and polyclonal aPL with serine protease (SP) enzymes that regulate hemostasis. Monoclonal human aPL cross-react with SP and bind to thrombin, activated protein C (APC), plasmin, tissue plasminogen activator (tPA), factor (F)IXa, and FXa [52–56], which share amino acid sequence homology at their catalytic sites. Several monoclonal human aPL inhibit inactivation of procoagulant SP and functional activities of anticoagulant/fibrinolytic SP [53, 55, 57, 58], and some aPL may recognize the catalytic domain of SP, leading to dysregulation of hemostasis and vascular thrombosis. Sera from patients with APS (including SLE-associated APS) bind different SP [55, 57].

Factor Xa has a central position in coagulation and mediates cellular inflammatory and anti-inflammatory processes [59]. Given its important position in coagulation and inflammatory pathways, plus recent addition of direct FXa inhibitors as alternative oral anticoagulants, interest has focused upon autoimmune-mediated regulation of FXa as well as other SP.

The work carried out at University College London examines the prevalence of IgG antibodies against FXa and associated SP, namely, thrombin (Thr), FXa, FVIIa, phosphatidylserine (PS)/FXa, and antithrombin (ATIII) in patients with APS and/or SLE as well as other autoimmune rheumatic disease (ARD) and healthy control (HC) groups. Furthermore, the effects of these antibodies upon the coagulant functions of FXa were studied. A significant difference occurred when anti-FXa IgG were present in patients with SLE (49.1%) or APS (33.9%) compared with ARD and HC where these antibodies were lacking ($p < 0.05$). Other anti-SP IgG were not specific to SLE and/or APS, with anti-Thr and anti-PS/FXa IgG being identified in other ARD and low levels of anti-FVIIa IgG found in all disease and HC groups.

Subsequent experiments utilizing purified anti-FXa-positive IgG revealed that the avidity of APS-IgG to FXa was higher than that of SLE-IgG. Furthermore, the greatest effects upon prolongation of FXa-activated clotting time (ACT) occurred with APS-IgG and inhibition of FXa enzymatic activity with APS-IgG followed by SLE-IgG when compared to HC-IgG. Antithrombin III inhibition of FXa was reduced by APS-IgG when compared to HC and SLE and did not correlate with binding to ATIII [60].

Inflammation is important in the pathogenesis of the APS through activation of complement and a family of G-protein-coupled receptors, known as protease-

activated receptors (PARs) [61] that are present on endothelial cells. Serine protease enzymes, including FXa, activate PARs.

Artim-Esen and Giles hypothesized that polyclonal IgG with anti-FXa positivity may alter PAR-mediated inflammatory as well as coagulant effects in patients with APS and/or SLE. To test this hypothesis, the researchers measured real-time intracellular calcium (Ca^{2+}) flux. They found a concentration-dependent induction of Ca^{2+} release by FXa that was significantly potentiated by APS-IgG compared to SLE/APS-IgG and to HC-IgG. Next, they examined the effects of a selective FXa inhibitor, antistasin, hydroxychloroquine, and fluvastatin in the presence or absence of patient IgGs. Treatment with all three drugs reduced FXa-induced and IgG-potentiated Ca^{2+} release.

Anti-FXa IgG isolated from patients with APS enhances both the enzymatic and cellular effects of FXa. Furthermore, FXa-mediated intracellular Ca^{2+} release in human umbilical vein endothelial cells is potentiated by IgG from anti-FXa-positive patients with APS and/or SLE. Further studies are now required to explore the use of IgG anti-FXa positivity as a novel biomarker and its potential to stratify treatment with FXa inhibitors.

The task force concluded that more data on the clinical utility of antibodies to FXa test are needed before any recommendation can be reached.

Annexin A5 Resistance Assay

This novel functional assay is based on the concept that annexin A5 has potent anticoagulant properties that result from its forming two-dimensional crystals over phospholipids, blocking the availability of the phospholipids for critical coagulation enzyme reactions [62–64].

Annexin A5 resistance is specific for APS-derived aPL (compared to aPL induced by syphilis) [65], correlates with risk of thrombosis and pregnancy complications [66, 67] and occurs in children with SLE [68], mainly in the presence of aPL [68, 69]. Resistance to annexin A5 anticoagulant activity correlates with aPL that recognize an epitope on domain I of β_2GPI [70].

The task force concluded that more data on the clinical utility of annexin A5 resistance assay test are needed before any recommendation can be reached.

Thrombin Generation Tests in Antiphospholipid Syndrome

Thrombin generation (TG) tests, also referred to as thrombography, are methods using fluorogenic substrates that allow measurement of total thrombin activity in vitro in response to low concentrations of tissue factor, summarized as the thrombin generation curve or thrombogram [71]. The thrombogram calculates several parameters: lag time, time to peak, peak height, and the total amount of thrombin

activity measured as the area under the curve, which is the endogenous thrombin potential (ETP) [72]. Activated protein C (APC) sensitivity of thrombin generation can be assessed either by adding APC or thrombomodulin. Activated protein C sensitivity can be estimated by an ETP ratio (APC sr) calculated by dividing the ETP measured in the presence of APC at a defined concentration by the ETP without APC. In addition, a dose-response curve of inhibition of ETP with increasing concentrations of APC (IC_{50}-APC) can also be performed. This index corresponds to the APC concentration that produces a 50% inhibition of ETP [73, 74].

Thrombin generation tests can be used for treatment monitoring. In the specific situation of APS, the intensity of anticoagulation can be assessed by means of the ETP. A recent multicenter study, the rivaroxaban in APS (RAPS) [75], included the percentage change in ETP from randomization to day 42 in both treatment arms (warfarin and rivaroxaban) as the primary outcome.

Thrombin generation assays have also been used to evaluate thrombotic risk. Quantitative measurement of LA activity by thrombin generation [76] correlates with thrombotic events [76].

Thrombin generation assays that indicate APC resistance (APCsr, IC_{50}-APC) are associated with thrombotic risk [77]. Protein C is a vitamin K-dependent glycoprotein that is cleaved by the thrombin–thrombomodulin complex into its activated form (APC). The mechanism of acquired APC resistance has been addressed by investigating the effect of aPL on the rate of activation of protein C or APC-mediated factor Va and factor VIIIa inactivation [78, 79], which might suggest interference by immune complexes of this anticoagulant pathway. Activated protein C resistance is associated with APS and is likely to be a mechanism leading to thrombosis. Thrombin generation-based tests indicate that patients with APS have an increased resistance to APC, the clinical significance of which is confirmed by at least one prospective cohort study, in which preliminary data indicate that patients with APC resistance have a higher risk for incident venous thrombotic events [80].

Quantitative assessment of LA activity in APS as well as APC activity can be obtained by thrombography, which constitutes an alternative for multiple assays and/or a functional assay for the effects of aPL in APS. The task force concluded that more data on the clinical utility of thrombin generation tests are needed before any recommendation can be reached.

International Society of Thrombosis and Hemostasis Antiphospholipid Antibody Standardization Subcommittee Solid-Phase Antiphospholipid Antibody Testing Guidelines

The International Society of Thrombosis and Hemostasis (ISTH), founded in 1954 by persons interested in thrombotic and bleeding disorders, with funding from the United States National Institute of Heart, Lung and Blood Institute, is an independent organization. It is not formally linked to the 15th International Congress on aPL or its workshops, but several members participate in both groups. An ISTH

subcommittee, aPL Standardization Subcommittee (ISTH-SSC), published recommendations on the detection of LA in 2009 that have proven useful in standardization of this assay [81]. In 2014 this subcommittee wrote recommendations that provide additional details and specifications for aCL and aβ_2GPI detection [82]. During the 15th International Congress on aPL, as part of a joint session organized by the Scientific Planning Committee of the Congress and ISTH-SSC, the most recent recommendations were discussed; these recommendations are endorsed by the task force.

Anticardiolipin and Anti-β_2-Glycoprotein I Antibodies

Anticardiolipin antibodies and aβ_2GPI are most commonly detected by ELISA. Recently, fully automated variations of the solid-phase ELISAs have been introduced [83–85] and tested for reproducibility [86]. These systems allow simultaneous detection of many types of aPL in a more rapid and less labor intensive way.

Although there has been some debate about the role of aCL (versus aβ_2GPI or other antibodies) in the diagnosis of APS, this ISTH-SSC recommends that aCL continue to be a laboratory criterion [1, 82]. Methodologically correct aCL assays with β_2GPI in the reagents have diagnostic value with similar sensitivities and specificities to aβ_2GPI [83, 84]. Since all assays use β_2GPI antigen, high correlation is observed between aCL and aβ_2GPI measured by the new automated systems as well as by standard ELISA [83, 87]. Demonstrating aCL and aβ_2GPI of the same isotype reinforces the probability of APS [81].

This is also an argument to keep IgG and IgM aCL and aβ_2GPI in the classification criteria [1, 82]. IgM aPL less often correlates with thrombosis than does IgG; for pregnancy morbidity, the role of IgM is unclear. Apart from a few studies for aCL and aβ_2GPI, all significant associations for IgM are also found with corresponding IgG antibodies, according to a recent review. Because absence of paired results of IgG and IgM for each patient hampered the evaluation of added value of IgM, the review did not answer how many APS patients might be missed if IgM testing were not done [88].

The ISTH-SSC does not recommend testing for IgA at this time [82] because the significance of IgA aCL and aβ_2GPI remains controversial. A recent report stated that testing positive for IgA aβ_2GPI resulted in a higher hazard ratio for APS compared to IgM aβ_2GPI [86]. Immunoglobulin A-positive patients often have at least one other criterion, suggesting that IgA testing may have less value in screening but may be used to confirm APS or to diagnose patients strongly suspected of having APS and who have negative tests for criteria aPL [3, 89].

In our choice for aPL solid-phase assays, we should be aware of inter-assay and inter-laboratory variability and the performance characteristics of the assays: a sample assigned positive in one assay does not automatically test positive in the same type of assay from a different manufacturer or in another laboratory. In this respect, the utilization of international units and polyclonal and monoclonal reference materi-

als for aβ$_2$GPI testing will contribute toward the much needed improvement of inter-laboratory and inter-assay agreement for aPL immunoassays [3]. Presently, the ISTH recommends performing all three assays (LA, aCL, aβ$_2$GPI) at the same time to increase diagnostic utility, with an integrated interpretation of LA, aCL, and aβ$_2$GPI and inclusion of an interpretative comment considering all three test results [82].

Antibodies to Domain I of β$_2$_Glycoprotein I

The 2014 SSC-ISTH recommendations did not include the anti-DI antibodies [82] because no commercial assays were then on the market, and more clinical studies were needed. Since then, research assays have been developed, and the association of anti-DI antibodies and thrombosis has been shown [90]. A commercial chemiluminescence immunoassay assay (CIA), using a recombinant DI bound on paramagnetic beads (the QUANTA Flash® β$_2$GPI-Domain I [Inova Diagnostics, San Diego, CA, USA]) now detects anti-DI. Several studies with this assay confirm high OR for thrombosis and the usefulness of anti-DI in risk stratification [91–96]. Also, the clinical significance of anti-DI and aβ$_2$GPI both detected with CIA technique shows high qualitative agreement ranging from 69 to 93% [91–96], much higher compared to the agreement in the first studies using an in-house anti-DI assay [14]. There is also a good correlation between both assays [92, 93, 95].

Reported OR for anti-DI differs and may be explained by the different detection platforms used to measure anti-DI and the limitation of calculating the anti-DI IgG's OR in patients positive for aβ$_2$GPI IgG. In evaluating antibody profiles measured by CIA, anti-DI IgG titers are higher in triple-positive patients [92, 93, 95]. Considering triple positivity to be strongly related to a more severe course of the disease, this reinforces the concept of anti-DI IgG positivity as a predictive tool in APS.

Although anti-DI are useful to identify the patients at highest risk for thrombosis, it is too soon to recommend replacement of aβ$_2$GPI testing by anti-DI testing. Studies that evaluate whether the anti-DI are an independent risk factor are limited in number. In multivariable logistic regression analysis, anti-DI IgG did not add diagnostic value to the current aPL panel (LA, aCL, aβ$_2$GPI) [92]. Comparable areas under the curve (AUC) for clinical complications were found for a model based on the current aPL panel with or without additional anti-DI IgG detection [92, 97]. Importantly, both aβ$_2$GPI and anti-DI were detected by the same CIA technique. The CIA aβ$_2$GPI assay probably detects mainly the antibodies targeting domain I, determined by the presentation of the protein on the solid phase. The good sensitivity of this aβ$_2$GPI for anti-DI antibodies was illustrated previously [98]. On the other hand, the CIA anti-DI assay is coated with recombinant β$_2$GPI domain I and the aβ$_2$GPI assay is coated with human purified β$_2$GPI [99]. Whether the cryptic epitope G40-R43 is exposed differently in the anti-DI and the aβ$_2$GPI IgG CIA assay should be further explored [21]. Before adapting the guidelines and adding anti-DI to the current aPL panel, more studies are needed on evaluating the value of the only available commercial anti-DI IgG assay comparing the performance of this assay with anti-β$_2$GPI assays

of other manufacturers. In assessing the additional value of the anti-DI assay, the type of aβ_2GPI assay to which the anti-DI assay is compared is crucial.

Non-criteria Solid-Phase Assays

With regard to the implementation of non-criteria aPL assays, for instance, antiphosphatidic acid, antiphosphatidyl-choline, antiphosphatidyl-ethanolamine, antiphosphatidyl-glycerol, antiphosphatidyl-inositol, antiphosphatidyl-serine, anti-prothrombin, anti-annexin A5, anti-protein S, and anti-annexin A2, in the diagnosis of APS, due to the lack of standardization and unconfirmed evidence about their clinical utility in APS patients, the ISTH does not recommend inclusion of other aPL in a standard test panel [3, 82, 100, 101].

Group Conclusion

This chapter updates the findings, conclusions, and recommendations presented during special sessions during the 15th International Congress on aPL (www.apsis-tanbul2016.org, North Cyprus, September 2016). Based on the available data, we suggest that testing for aPS/PT can contribute to the diagnosis of APS and to the assessment of risk of thrombosis. Regarding antibodies to domains of β_2GPI, anti-DI displays higher specificity but lower sensitivity in comparison to the assay with the whole molecule. It is too soon to recommend replacement of aβ_2GPI testing by anti-DI testing. Antibodies against DIV-V of β_2GPI show lower specificity for APS or systemic autoimmune conditions than do anti-DI antibodies. Due to the unavailability of the assay to detect these antibodies, no recommendations are given on this subject. As per previous recommendations, as positive IgA aCL and IgA aβ_2GPI are usually associated with other aPL, their utility is restricted to those patients with a strong suspicion of APS but negative aPL tests. With regard to other tests, such as aPHL, anti-factor Xa, and annexin V resistance, and thrombin generation assays, more data on their clinical utility are needed before any recommendation can be reached.

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Chapter 9
Disease and Risk Measurement Criteria in Antiphospholipid Syndrome

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Introduction

Quantifying the risk of thrombosis or pregnancy morbidity in patients with antiphospholipid antibodies (aPL) switches the concept from thinking of aPL as diagnostic antibodies to considering them to be risk factors for clinical events.

Current clinical wisdom accepts the following points regarding serologic risk prediction: triple aPL-positive (lupus anticoagulant [LA], anticardiolipin antibody [aCL], and anti-β_2-glycoprotein-I antibody [aβ_2GPI]) patients have higher thrombosis risk than do patients with one or two positive tests; moderate/high titers (>40 U or >99th percentile) correlate better with clinical manifestations than do low titers; and immunoglobulin G isotype has a higher predictive value than does IgM.

Only LA, aCL, and aβ_2GPI are included in the updated Sapporo antiphospholipid syndrome (APS) classification criteria [1]. In these criteria, the heterogeneous nature of an aPL profile is not represented, and the criteria do not offer risk stratification based on non-aPL risk factors. Other aPL tests, for instance, antiphosphatidylserine/prothrombin (aPS/PT) antibody, may be useful in the scoring systems described below.

Two scoring systems, aPL score and the global APS score (GAPSS), have been proposed to predict risk and to stratify patients [2, 3]. These systems accept that recurrent thrombosis risk may be predicted from aPL immunoglobulin subtypes, titers, and profiles. Antiphospholipid antibody profiles can be quantitated with the aPL Score, which can be used both to diagnose APS and to predict thrombosis in autoimmune diseases [2]. The GAPSS [3] takes into account aPL profile (both criteria and non-criteria aPL) and conventional cardiovascular risk factors and quantifies risk of thrombosis/obstetrics events. Initially developed and validated in patients

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with SLE, GAPSS has been studied in patients with primary APS (PAPS) [4] and in an independent cohort of Japanese patients with autoimmune diseases [5]. Another study confirmed that GAPSS predicts thrombosis in aPL patients [6].

Large, prospective cohort studies of patients with different clinical subsets of APS confirm that APS conveys high morbidity and mortality and has severe socioeconomic impact. The long-term prognosis in patients with APS is influenced by recurrent thromboses. Other organ damage is caused by mechanisms that are only partially understood. To understand these factors better, a specific damage index for APS (DIAPS) has been developed and validated; it is described below [7].

Health-related quality of life (HRQoL) is an outcome measure that can assess the burden of the disease from a patient's perspective and that can be used in therapeutic studies and other clinical trials. Damage caused by thrombosis, for instance, gangrene requiring amputation, decreases HRQoL [8]. It, too, is described below.

Antiphospholipid Antibody-Related Risk Measurement

In 1996 Finazzi et al. [9] suggested that high-titer aCL is an independent predictor of thrombosis. Pengo et al. [10] found that LA and aβ_2GPI were independent risk factors and that triple positivity (LA, aCL, aβ_2GPI) was a stronger risk factor for thrombosis. Of 370 non-APS patients with IgG and/or IgM aCL or LA, Gresele et al. reported that 8% developed thrombosis in a follow-up period of 59 months and that high-titer IgG aCL independently predicted this event [11].

A growing body of evidence supports that different aPL profiles may imply different thrombotic risks [9–11]. However, while aPL appear to be pathogenic in several in vitro and in vivo models [12, 13], their interactions with blood procoagulant activities have still to be fully understood [14–18]. Monoclonal antibodies or IgG from patients with APS directly stimulate procoagulant activity in monocytes and endothelial cells in vitro; enhanced expression of coagulation proteins and adhesion molecules is a function of antibody concentration [15–18]. Similar phenomena occur for aPS/PT, aCL, and aβ_2GPI. These data suggest that quantitative rating of thrombotic risks from the detailed analysis of aPL profiles is possible.

Antiphospholipid Score (aPL-S)

Otomo et al. developed an antiphospholipid score (aPL-S) to quantify the risk of thrombosis [2] and to guide therapy. Their study analyzed two cohorts of autoimmune patients from Hokkaido University Hospital. The first, "backward," cohort contained 233 consecutive patients examined in 2006. The second, "forward," cohort had 296 patients who visited the clinic from 2002 to 2003 and were followed for two or more years. Lupus anticoagulant positivity was diagnosed with three clotting tests (activated partial thromboplastin time [aPTT], dilute Russell viper venom

time [dRVVT], and kaolin clotting time [KCT]) with mixing and phospholipid neutralizing assays, according to the guidelines suggested by the LA/aPL Scientific and Standardization Subcommittee of the International Society on Thrombosis and Haemostasis [19]. Anticardiolipin antibodies (IgG and IgM), $a\beta_2$GPI (IgG and IgM), and aPS/PT (IgG and IgM) were assayed by enzyme-linked immunosorbent assay (ELISA) [2, 20–22]. A second analysis separated patients with high antibody levels from those with lower levels. To define aPL-S, odds ratios [ORs] for thrombosis and/or pregnancy morbidity for each aPL test was calculated using the backward cohort and this formula: aPL-S = 5 Xexp ([OR]-5)/4 (Table 9.1). Another analysis, the partial aPL-S, used only the criteria tests, IgG/IgM aCL, IgG/IgM $a\beta_2$GPI, and LA (aPTT and dRVVT).

The prevalence of APS manifestations in patients with aPL-S = 0 was 10%, for those with 1–9, 26%; for those with 10–29, 29%; and for those with >30, 56%. Similar results were observed with the partial aPL-S. Receiver-operating characteristic [23] curves for APS diagnosis using aPL-S, partial aPL-S, and revised

Table 9.1 Relative risk of clinical manifestations of APS for each aPL test

Test	Cutoff	Sensitivity (%)	Specificity (%)	OR (95%CI)	aPL score
APTT mixing confirmation test, ratio	>49s	39.1	89.3	5.36(2.53–11.4	5
	>1.3	19.6	95.2	4.81(1.79–12.9)	2
	>1.1	30.4	90.9	4.38(1.96–9.76)	1
KCT mixing	>29s	45.6	88.8	6.64(3.17–13.9)	8
dRRVT mixing confirmation test, ratio	>45s	28.2	90.9	3.93(1.74–8.88)	4
	>1.3	17.4	94.7	3.72(1.38–10.1)	2
	>1.1	28.3	90.4	3.7(1.65–8.27)	1
IgG aCL, GPL high titers	>30	15.2	98.4	11(2.72–44.5)	20
IgG aCL, GPL medium/low titers	>18.5	19.5	94.6	4.31(1.63–11.3)	4
IgM aCL, MPL	>7	6.5	96.3	1.79(0.45–7.22)	2
IgG $a\beta_2$GPI, units high titers	>15	23.9	98.4	19.3(5.11–72.7)	20
IgG $a\beta_2$GPI, units medium/low titers	>2.2	30.4	92.5	5.4(2.35–12.4)	6
IgM $a\beta_2$GPI, units	>6	8.7	91.4	1.02(0.32–3.20)	1
IgG aPS/PT, units high titers	>10	19.6	97.8	11.1(3.25–38.1)	20
IgG aPS/PT, units medium/low titers	>2	28.3	95.7	8.81(3.39–22.9)	13
IgM aPS/PT, units	>9.2	6.5	98.9	6.45(1.05–39.8)	8

APS antiphospholipid syndrome, *aPL* antiphospholipid antibody, *OR* odds ratio, *95%CI* 95% confidence interval, *APTT* activated partial thromboplastin time, *KCT* kaolin clotting time, *dRVVT* dilute Russell viper venom time, *aCL* anticardiolipin antibody, *GPL IgG* phospholipid units, *MPL* IgM phospholipid units, *$a\beta_2$GPI* anti-β_2-glycoprotein-I antibody, *aPS/PT* anti-phosphatidylserine antiprothrombin antibody

Sapporo criteria all showed a hyperbolic pattern, implying that aPL-S is a potential quantitative marker for APS diagnosis.

Regarding predictive ability, the aPL-S and partial aPL-S were higher among patients in whom thrombosis developed than among those without thrombosis (median score 5.5 in aPL-S with thrombosis vs. 0 and 5.5 vs. 0 in partial aPL-S). Odds ratios (OR) for new thrombosis in patients with aPL-S of ≥10 and ≥30 were 2.9 and 5.3, the positive predictive values 20% and 31%, and the negative predictive values 92% and 92%.

In a multivariate Cox regression tests that included age, gender, glucocorticoid treatment, hypertension, hyperlipidemia, diabetes, SLE, or rheumatoid arthritis, an aPL-S of ≥30 was an independent risk factor for thrombosis (OR 3.144 [95% CI 1.383–7.150], $p < 0.006$) [23].

Global Antiphospholipid Syndrome Score (GAPSS)

The global APS score (GAPSS) (Table 9.2) was first developed and validated in a large cohort of patients with SLE, divided into two statistically independent sets by a computer-generated randomized list [24]. According to this model, risk assessment quantification was based on the computation of independent factors for thrombosis and pregnancy loss. The variables identified by the multivariate analysis to be independently related to thrombosis or pregnancy morbidity include criteria (LA, aCL, and aβ_2GPI) [1] and non-criteria (antiprothrombin antibody, aPS/PT) aPL [25] and cardiovascular risk factors of arterial hypertension and hyperlipidemia. Global APS score gives these factors weights proportional to the β-regression coefficient values: five points for IgG/IgM aCL, four for IgG/IgM aβ_2GPI and LA, three for IgG/IgM aPS/PT and hyperlipidemia, and one for arterial hypertension. In a prospective assessment of 51 SLE patients followed for a mean 34 months, an increase of >3 GAPSS points the best predicted risk for vascular events (OR 48 [95% CI

Table 9.2 The global antiphospholipid syndrome score (GAPSS)

Factor	Point value
Anticardiolipin IgG/IgM	5
Anti-β_2-glycoprotein-I IgG/IgM	4
Lupus anticoagulant	4
Antiphosphatidylserine/prothrombin (aPS/PT) IgG/IgM	3
Hyperlipidemia	3
Arterial hypertension	1

The GAPSS scoring system is derived from the combination of independent risk for thrombosis and pregnancy loss and accounted for multiple factors, including the patient's aPL profile, conventional cardiovascular risk factors, autoimmune antibody profile, and thromboprophylactic drug use. The GAPSS can be calculated for each patient by adding the points corresponding to the different risk factors, weighted as shown in this table

6.90–333.85, $p = 0.0001$]) [26]. In 62 consecutive patients with PAPS, higher GAPSS values occurred in patients with thrombosis than with pregnancy morbidity [4]. Patients with recurrent thrombosis had higher GAPSS than did those without recurrences. Patients with GAPSS ≥11 points had an 18-fold increase in risk of recurrence, conclusions confirmed by Oku et al. [5] in a retrospective cohort of 41 APS and 241 control patients. Higher GAPSS values occurred in patients who had experienced arterial and/or venous thrombosis. Zuily et al. evaluated the power of GAPPS to predict thrombosis in a prospective multicenter cohort study [6]. Of 137 consecutive patients with aPL or SLE (mean age 43.5) followed for a mean of 43 months, patients who experienced thrombosis had a mean GAPSS score of 10.9 compared to those with no thrombosis (GAPSS 8.2). In multivariate analysis, GAPSS >16 was the only predictor of thrombosis (OR 6.2 (95% CI 1.70–22.40)).

Antiphospholipid Antibody-Related Damage Measurement

In an early study that addressed functional outcomes after 10 years, Erkan et al. reported that 38% of PAPS patients had reduced functional status as a result of hemiparesis, dementia, quadriplegia, or cardiac compromise [27]. Shah et al. reported that 9 of 31 (29%) patients with APS had further thromboses during 10 years' follow-up [28]. A systematic review based on a multicenter European survey reported increased mortality rates (5.3–6.7%) of APS patients, primarily due to arterial thrombosis [29]. Quality of life (QoL) issues related to obstetrical complications are also important to long-term prognosis [30].

The SLICC/ACR damage index (SDI) measures cumulative organ damage but lacks specificity for APS. Grika et al. [31] as well as Barbhaiya et al. [32] analyzed the usefulness of SDI in patients with aPL, APS, SLE, and other autoimmune conditions. They found that SDI increases with damage but were unable to identify organ damage directly related to aPL, suggesting that SDI overestimates damage related to lupus and minimizes that related to aPL.

A damage index in APS (DIAPS) has been designed to include APS-specific items, all thrombotic, not considered in SDI [7]. DIAPS has solid methodological steps [33]. It measures irreversible damage in different systems affected by APS. DIAPS consists of 37 items and can be completed by an untrained physician in 20 min. The construction and validation of the questionnaire are shown in Fig. 9.1. A Delphi panel performed with an international group of experts showed a high reliability in 94% of its items.

DIAPS is the first published report of the development and validation of a questionnaire to assess aPL-related organ damage in patients with APS [7]. In its initial validation, DIAPS was applied to 156 patients with thrombotic APS from four Latin American countries. A key step was the simultaneous application of a generic quality of life instrument, EuroQoL. DIAPS had a high internal consistency. The EuroQol domains of mobility, pain/discomfort, health status, usual activities, and self-care correlate with the global DIAPS score. Ugolini Lopes et al. [34] compared SDI and DIAPS in 93

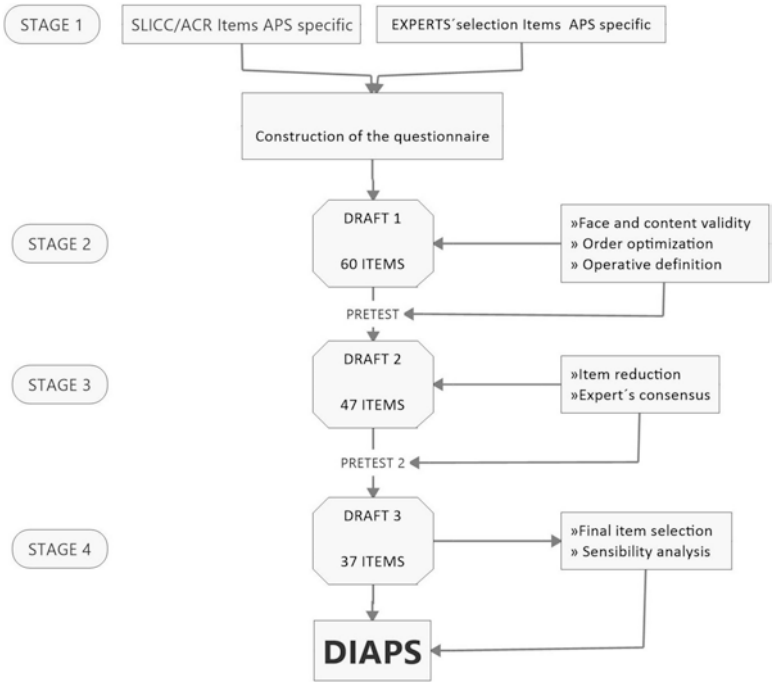

Fig. 9.1 Damage index in antiphospholipid syndrome (*DIAPS*) development and validation

PAPS patients and found correlation between duration of illness and higher scores with both indices; DIAPS seemed to be a better measure of severity in APS.

Non-thrombotic manifestations of the syndrome such as livedo reticularis/racemosa, multiple sclerosis-like CNS disease, and diffuse pulmonary hemorrhage were not included in DIAPS, although "non-criteria" manifestations are part of the wide spectrum of APS [35] and many common manifestations of APS are not thrombotic. The impact that obstetric morbidity of APS and aPL have on chronic damage remains unknown.

Suggestions regarding items that may improve the reliability and accuracy of DIAPS include need of an operational definition of pulmonary hypertension and neuropsychiatric manifestations (such as cognitive dysfunction), and of adding damage secondary to bleeding from excessive anticoagulation [36], alveolar hemorrhage, adrenal hemorrhage, severe thrombocytopenia, hypoprothrombinemia, and catastrophic APS. Further, because DIAPS items are binary, weighing each item may give DIAPS more clinical relevance. Future research requires long-term follow-up of the patients to establish sensitivity, specificity, predictive values, sensitivity to change, and reliability. DIAPS was developed and validated using specific aPL manifestations; other manifestations should be added. Because DIAPS was developed based on Latin American patients, further studies should include additional populations.

Antiphospholipid Antibody-Related Quality of Life Measurement

Quality of life includes health as well as other domains, such as jobs, schools, culture, and values; it comprises both physical and mental health. Health-related quality of life (HRQoL) predicts mortality and morbidity [37] and is impaired in patients with history of venous thrombosis [38] or of SLE [39]; SLE-related disease activity and damage over time predict poor HRQoL [40]; recent studies ask whether APS or aPL impair HRQoL [41].

Medical Outcomes Study Short-Form 36 (MOS-SF-36)

Health-related quality of life can be assessed using the Medical Outcomes Study Short-Form 36 (MOS-SF-36) [42]. This is a self-administered questionnaire that contains eight dimensions: bodily pain (BP), general health (GH), mental health (MH), physical function (PF), role emotional (RE), role physical (RP), social function (SF), and vitality (VT). It is a generic, reliable, and valid measure for HRQoL. A score between 0 (worse) and 100 (best) is calculated for each dimension. Two subscales can be computed as different weighted sums of dimension scores, i.e., a mental (MCS) and a physical component summary (PCS) score to obtain a mean of 50 with a standard deviation of 10 in a healthy general population [43]. A difference of more than five points in one of the eight dimension scores is considered as clinically significant [43, 44]. The validity and reliability of MOS-SF-36 questionnaire in APS have not been assessed.

EuroQol 5 Dimensions (EQ-5D)

Health-related quality of life can also be assessed using the EQ-5D (EuroQol 5 dimensions), a generic, standardized, and validated questionnaire that measures five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has three levels: no, some, or extreme problems. EQ-5D is short and multilingual [45]. It shows consistent results in different countries and in many chronic diseases, including rheumatic [46, 47]. The EQ-5D is a useful surrogate to calculate the economic impact of disability. Although specific scales exist for SLE patients, none is yet available for APS patients.

Three studies investigated HRQoL in APS patients. A Brazilian case-control study of 30 PAPS patients and 40 gender-, age-, and race-matched healthy controls concluded that APS patients have lower values in all dimensions of the MOS-SF-36 [48]. A cross-sectional survey of 270 members of the Hughes Syndrome Foundation (60% from the UK, 25% from the USA, and 15% from elsewhere) asked patients to

respond to an online questionnaire that included the MOS-SF-36, demographic, and disease-specific characteristics and self-reported major manifestations of APS [49]. In all eight domains of the MOS-SF-36, the mean scores were lower in PAPS and other autoimmune disease associated APS patients than in British and US normative data. A French multicenter study used MOS-SF-36 in 115 aPL-positive and SLE patients [8]; scores were dramatically impaired in all but the PF dimension. All dimension scores were impaired in the 25–54-year age group; patients 45–54 years old had the greatest impairment. In patients18–24 and 55–64 years old, three out of eight dimensions were lower. No dimension scores were lower in patients aged 65–74 years. Health-related quality of life in men was impaired in all dimension scores, while in women only five dimensions were lower. More HRQoL dimensions were impaired in women 25–34 years old and more in men 35–44.

Two studies asked whether aPL impacts QoL in SLE patients; they yielded conflicting results. Balitsky et al. in a cross-sectional study in 2011 [50] did not find that APS or aPL negatively impacts HRQoL measures (PCS and MCS scores) but APS in SLE patients did decrease HRQoL. Mental component summary score in aPL-positive SLE patients were lower than in aPL-negative SLE patients. Although the main hypothesis was that thrombosis results in impairment, Balitsky et al. did not confirm this hypothesis.

A different study found that arterial thrombosis but not venous thrombosis was associated with impaired HRQoL [51]: myocardial infarction affected physical dimensions; and ischemic stroke, to a lesser extent, affected mental dimensions. When age, arterial thrombosis, and any cardiovascular risk factors were entered in a multivariate model, arterial thrombosis was the only factor associated with an impaired PCS or MCS score, similar to prior studies in the general population [51]. Furthermore, arterial thrombosis impaired HRQoL independently of cardiovascular risk factors, not previously reported in this population. While patients with post-venous thrombotic syndrome in a general population have impaired HRQoL [52], APS patients, who were younger than the general population patients, may not have had sufficient time to develop severe postthrombotic syndrome.

Health-related quality of life measures do not correlate well with the SLEDAI [41], possibly because more disease may not be associated with worse HRQoL. Among SLE patients with or without aPL, SLEDAI values correlate negatively with only one MOS-SF-36 dimension (PF) [51].

Regarding APS without SLE, Amigo et al. found that DIAPS correlates with HRQoL, assessed by EQ-5D [7], particularly with the following domains: mobility, pain/discomfort, health status visual analog, usual activities, and self-care. Costa et al. showed that fibromyalgia in PAPS patients is associated with a dramatic impairment of all dimensions of MOS-SF-36 questionnaire except SF and MH [48].

To date, no data are available regarding the possible psychological impact of knowing that one has APS or of long-term antithrombotic treatment. Furthermore, we do not know whether HRQoL varies during life, especially after a thrombotic event, and how it may recover.

The development of a dedicated scale for the measurement of HRQoL in APS patients will be necessary to evaluate all the spectrum of APS. The use of HRQoL

questionnaires should be considered in everyday patient care. HRQoL questionnaires, as a surrogate for clinical outcomes, together with thrombosis recurrence rate and survival, will be needed in future APS research.

Group Conclusion

Evaluating risk profile in patients with aPL is an unmet need. Both the aPL score and the GAPSS are useful tools to quantify the risk of thrombosis or pregnancy morbidity in aPL-positive subjects, i.e., aPL are not diagnostic antibodies but risk factors for clinical events. Some aPL-related manifestations carry a worse prognosis than do others, and permanent damage occurs in some but not all organs. A specific damage index for APS (DIAPS) has been developed and validated. The consequences of damage related to thrombosis cause a decrease in HRQoL.

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Chapter 10
Neuropsychiatric Manifestations of Antiphospholipid Syndrome

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Introduction

Since the first publication on antiphospholipid syndrome (APS) [1], many neuropsychiatric manifestations have been described. However, because of limitations of studies and the coexistence of confounding factors, such as systemic lupus erythematosus (SLE), associations between neuropsychiatric manifestations and APS remain unclear. The current (Revised Sapporo or Sydney) clinical classification criteria for APS include major thrombotic manifestations [2], but others, "minor" manifestations commonly seen in routine clinical practice, are not considered. In this chapter, we summarize current knowledge on neuropsychiatric manifestations in APS, remaining questions, and research directions in the field.

Stroke and Transient Ischemic Attack

Epidemiological and Clinical Findings

Cerebral ischemia is the only neurological manifestation included in the classification criteria for APS. The association between this manifestation and aPL has been recognized since the very early studies, with a few negative reports coming from studies that included mostly isolated, low titer anticardiolipin antibody (aCL) positive patients [3, 4]. Antiphospholipid syndrome patients with stroke are 10 years younger than a global population of patients with stroke, and they have fewer cardiovascular risk factors [5].

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Data from the Euro-APS, the largest cohort of APS patients, show a prevalence of 31% for stroke and transient ischemic attack (TIA) in patients diagnosed as having APS [5]. A recent review states that antiphospholipid antibodies (aPL) occur in 13.5% of patients with a first stroke [6]. Lupus anticoagulant (LA) conveys a 43-fold increase in the risk of a first stroke in a population of young women [7]. This risk is additive when other risk factors, such as smoking and contraceptive pills, exist [7–9]. In aPL-positive SLE patients, valvulopathy has been closely associated with stroke [10].

Because of the increased risk of recurrence after a first stroke in the following year, it is important to diagnose APS [10]. In a recent large-scale study including 1867 young patients (<45 years) after a first ischemic stroke, the 10-year risk of recurrence was three times higher in patients with aPL compared to those without aPL [11].

Neuroimaging Findings

Neuroimaging abnormalities occur with high frequency in aPL-positive patients [12]. Ischemic lesions in APS are similar to those found in any patient with ischemic cerebral infarction, with no specific features. White matter hyperintensity lesions (WMHL) are frequent in magnetic resonance imaging (MRI) of individuals with aPL; their clinical significance is controversial as WMHL also occur in asymptomatic subjects [13, 14].

Pathophysiology

Mechanisms underlying cerebral infarction are complex and only partly understood. In situ thrombosis is more frequently observed than is embolism from a cardiac source. The procoagulant effects of aPL are well documented in mice immunized with β_2-glycoprotein-I (β_2GPI) [15–17]. Endothelial dysfunction induced by aPL is an important mechanism of thrombus formation through endothelial cell production of adhesion, pro-inflammatory, and procoagulant chemokine/factors [18]. Interactions between aPL and cell receptors, such as toll-like receptors and annexin A5, are under investigation [19].

Early atherosclerotic vascular damage occurs in APS and may contribute to arterial thrombosis. An increase in intima-media thickness or pulse wave velocity, measures of atherosclerosis, correlates with a decrease in paraoxonase antioxidant activity, thus increasing production of oxidized LDL (oxLDL), a component of atheromatous plaque [20, 21]. Oxidized LDL-β_2GPI complexes have been found in the arterial wall in APS animal models [22], and aPL interact with oxLDL [23, 24]. These complexes are thought to activate release of inflammatory cytokines and enhance recruitment of immune cells into arterial walls, thus contributing to premature atherosclerosis.

Cognitive Dysfunction and Dementia

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

Although cognitive dysfunction is not included in the classification criteria, it is frequently seen in APS patients, suggesting a link between these two entities. The reported prevalence of cognitive dysfunction in aPL carriers ranges from 19% to 40% [25–27] and from 42% to 80% in primary APS [28, 29]. A longitudinal analysis of 800 elderly subjects showed that cognitive dysfunction and motor decline are associated with brain infarct and aPL [30]. Similar findings occur in younger subjects and in asymptomatic subjects when they are compared to healthy age-gender-matched and education-matched controls [28]. Tektonidou et al. reported that cognition disorders are independent of history of neurologic involvement, including stroke, suggesting that mechanisms other than thrombosis may be involved [28].

A relationship between aPL titers and cognitive dysfunction has also been described [26]. Lower performance in attention, learning and memory, and executive and visuomotor skills was observed in the 85 patients with high titer aCL compared to 58 patients with moderate titer aCL. Cognitive dysfunction has no known association with demographic or clinical characteristics of aPL carriers; it is correlated with age and with livedo reticularis in APS [28]. Whether cognitive dysfunction correlates with ischemia in APS remains unknown.

While some reports suggested that aPL were nonspecifically elevated in elderly subjects, two studies suggest a possible association between aPL and dementia [31, 32]. Juby et al. demonstrated that aCL were not increased in healthy elderly subjects (frequency of aCL is 0%) but was increased in patients with multiple infarct dementia [31]. When Mosek et al. compared aPL positivity in 87 patients with dementia and 69 healthy controls, they found that 6% of the demented had aCL > 20 GPL, but none of the controls did [32]. Of note, all demented aPL-positive patients in this study had Alzheimer-type dementia.

A large cohort of 1000 APS patients reported a prevalence of dementia of 2.5% [5], which increased to 56% in older (mean age 65 years) APS patients [33].

Neuroimaging Findings

Data on imaging in aPL carriers are scarce. One study on 30 aPL carriers with cognitive dysfunction or dementia reported a high frequency of WMHL [34]. A prospective cohort of asymptomatic subjects with low titer aCL reported no association between abnormal neuroimaging findings and aPL [35]. In APS brain imaging abnormalities are frequent [13], with WMHL, varying from small focal to diffuse lesions, and related to ischemic and inflammatory lesions [36], being the most common. These abnormalities are nonspecific and their prognostic value is unclear.

Using functional MRI, and comparing aPL-positive patients to controls, Kozora et al. found abnormal activity in bilateral frontal, parietal, and temporal areas, areas of working memory and executive function [37].

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

An association between aPL and cognitive dysfunction occurs in SLE patients, with a reported prevalence of 21–54% in SLE patients with aPL, compared to 4–7% in those without aPL [38–45]. Mikdashi et al. demonstrated that aPL independently predicts cognitive dysfunction in 130 SLE patients [41]. Conti et al. showed that this association is specific to aPL and that other autoantibodies detected in SLE patients are not associated with this disorder [45]. Moreover, the correlation between aPL and the severity of the cognitive decline supported the hypothesis that links aPL with cognitive dysfunction [38, 41]. A high frequency and a similar pattern of cognitive dysfunction have been reported in both SLE without aPL and APS patient groups [27, 28]. Kozora et al. found cognitive dysfunction in 40% (8/20) of the non-SLE aPL-positive patients compared to 60% (12/20) of aPL-negative SLE patients, with immediate and delayed verbal memory being the most impaired functions [27]. In contrast, in a study limited by a small sample size (n was 51) and inclusion of low titer aCL, Hanly et al. found no association between aPL and cognitive impairment [46]. In addition, a study from a Canadian cohort of 1000 SLE patients found that when strokes were excluded from the definition of neuropsychological manifestations, aPL were no longer associated with neurological impairment [47].

Neuroimaging Findings

Neuroimaging abnormalities found in SLE aPL-positive patients are similar to those found in non-SLE aPL-positive patients. Antiphospholipid antibodies are associated with WMHL on MRI [48]. As in non-SLE patients, the prognostic value of these lesions is still uncertain, and some studies report no correlation between neuroimaging findings and severity of cognitive dysfunction [27].

Pathophysiology

There is growing experimental evidence of direct immunopathologic effect of aPL on the brain. Animal models show that after three months of exposure to aPL, mice display memory impairment, anxiety, and hyperactivity (compared to aPL-induced

thrombosis that occurs in hours or days) [49, 50]. On microscopic examination of mice brains, mononuclear inflammatory infiltrate and a reduction of dendritic complexity in hippocampal CA1 neurons were found [51]. The authors hypothesize that an inflammatory process may increase the brain blood barrier permeability allowing aPL binding to brain cells, an effect observed in vitro [51]. In mice, this binding correlates with neurological abnormalities that are thought to model cognitive dysfunction [52, 53]. In a mouse model of Alzheimer's disease, aPL but not other inflammatory triggers exacerbate the accumulation of mature amyloid plaques and cognitive decline [54]. While the spectrum of brain abnormalities and cognitive impairment have been described in children born to mothers with APS, the effect of aPL on fetal brain is not yet well characterized [55, 56].

Migraine

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

Prevalence of migraine is 18–40% of aPL-positive patients [5, 34, 57]. The lack of a consistent definition of headache and migraine and the variabilities in study protocols, for instance, patient self-report vs. direct questioning, may explain the wide range. The association between aPL and migraine is still debated. Some studies report elevated aPL in patients with migraine (12–14%) when compared to healthy controls (0–4%) [58, 59], whereas others do not [60–65]. The strongest study with respect to methods and sample size shows that 12% of 284 patients with migraine have aPL, compared to 3% of 225 healthy controls [59].

In APS patients, migraine is associated with the LA and history of stroke [66, 67]. However, migraine also occurs in patients without history of brain ischemia or ischemic lesions in brain imaging, suggesting the involvement of inflammatory and immune process [34]. Pardos-Gea et al. performed echocardiograms on patients with newly diagnosed APS. Patients with valve disease were more likely to have arterial thrombosis, livedo reticularis, and migraine [68] when compared to APS patients without valve disease. Krause et al. also reported that migraine is associated with valve disease [69]. In a subsequent cluster analysis of APS patients, Krause et al. found an association among recurrent pregnancy loss, intrauterine growth restriction, migraine, and epilepsy [70].

Recently, Islam et al. performed a meta-analysis including nine studies. In this work, which was presented during the 15th International congress on aPL, the authors showed a significant association between migraine and aCL or aβ_2GPI, with relative risks of 6.3 and 2.74, respectively, and no association with the LA [71]. Given the methodological limitations of previous studies, the 14th International Congress on aPL Task Force on Non-criteria aPL Manifestations concluded that

there is no strong evidence for a causal relation between migraine and APS [65]. This task force recommended further studies using consistent definition of headache and migraine and the fulfillment of laboratory criteria in studies to come. Those studies are still awaited.

Neuroimaging Findings

The relationship between MRI-visualized WMHL and elevated aPL was evaluated in 102 migraine patients. In this study, WMHL were observed in 26% of the patients with migraine compared to 6% of the healthy controls. None of those patients had elevated aPL suggesting that WMHL are common in migraine, and the link between those WMHL and aPL in migraine is unclear [72].

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

Migraine and headaches are often reported in SLE patients with aPL/APS. No clear distinction was performed between unspecific headache and migraine in some reports, whereas others used the international migraine definitions. When migraine was well defined according to international headache classification criteria [73], a prospective study of 103 SLE patients reported no association between aCL and migraine [74]. Three other prospective studies reported an increased risk of migraine in aPL-positive SLE patients (OR 4.2–7.5) [38, 75, 76]. A different study found that, in contrast to primary APS patients, there was no association between valve disease and migraine in SLE patients [69]. Controversial data have been reported on the association between headache or migraine and cumulative organ damage and disease activity in SLE patients [75, 77]. Clearly, discrepancies between studies are mainly due to inconsistent definitions of headache; unspecific headaches seem to be frequent in SLE patients that are unrelated to disease activity or specific autoantibodies such as aPL. Migraine might be associated with aPL in SLE patients, but more studies are needed to confirm this association and to properly assess the causal relation between aPL and migraine.

Neuroimaging Findings

No specific neuroimaging pattern has been described in migraine associated with aPL in SLE patients.

Pathophysiology

The pathological mechanisms involved in aPL/APS patients with migraine remain poorly understood. Even if an increase frequency of migraine has been reported in patients with a history of stroke, migraine is also observed in aPL/APS patients without ischemic events. Possible common pathogenic mechanisms, such as platelet activation, serotoninergic and dopaminergic system involvement, and complement activation, may be hypothesized. However, data are insufficient to assess these mechanisms.

Epilepsy and Seizures

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

Seizures, described as part of the syndrome in APS patients since the early descriptions, are not part of the current APS classification criteria. Only few studies report the prevalence of seizures in APS patients without SLE, ranging from 3.2% to 10% [5, 70, 78–80], being ten times higher than that of the general population [81]. The association between aPL and seizure/epilepsy is debated, with positive [82, 83] and negative reports [84, 85].

Other clinical manifestations may accompany seizures in patients with APS. In a retrospective cohort of 173 primary APS and 134 secondary APS patients, Krause et al. reported an association of thrombocytopenia and epilepsy [86]. Other clinical factors were preexisting ischemic lesions, livedo reticularis, smoking, and heart valve disease [80].

The 14th International Congress on aPL Task Force on "non-criteria" APS manifestations highlighted the conflicting results and the need to further study this potential association by taking into account the presence of brain infarction by including neuroimaging and adjust analysis for the occurrence of stroke as potential causal factor for seizure [65].

An American study, based on a medical insurance registry population that included more than two million subjects, confirmed a 9.5-fold increase [95% CI 8.1–11.1] in the risk of seizures in APS [83]. This study has limitations, including lack of information on the diagnostic criteria for APS and information on other relevant confounding factors, such as a history of prior stroke. In addition, a recent meta-analysis by Alam et al. presented in the 15th international congress on aPL reported a significant association between seizure and aCL but not with aβ_2GPI [87].

Neuroimaging Findings

There are no specific lesions for seizures in aPL/APS patients without SLE.

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

Compared to primary APS patients, SLE-associated APS patients have a higher increased prevalence of seizure/epilepsy (13.7–18.4% versus primary APS 6–8.4%) [67, 80]. Shoenfeld et al. reported SLE as an independent factor seizure in APS [80]. The association of aPL with seizures is higher in SLE patients [75, 76, 88, 89]. In contrast, a large study from the SLICC cohort consisting of 412 SLE patients enrolled at diagnosis and prospectively followed showed no association between seizures and aPL [90]. Recently, Hawro et al. [76] reported a strong association with aβ2GPI (OR 11.25 [2.01–62.97]) and the LA (OR 6.75 [1.16–39.6]). Despite its small sample size (*n* of 57), this study was methodologically strong, all patients fulfilled current APS Classification Criteria.

Pathophysiology

An autopsy report from a patient with SLE and seizures showed multiple infarctions in cerebral cortex [91]. However, non-thrombotic direct effect of aPL inducing seizures has been confirmed. Seizures can occur in aPL-positive patients who do not have ischemic lesions in neuroimaging [92]. Functionally, aPL induce a rapid depolarization and increase the membrane permeability of neurons [93]. In a model that uses snail neurons, aCL inhibits gamma-aminobutyric acid (GABA)-receptor complex in a dose-dependent manner without altering the glutamate responses, suggesting that this mechanism may induce seizures by enhancing neuronal excitability [94].

Multiple Sclerosis-Like Syndrome (MS) and Myelitis

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

Symptoms mimicking MS, such as transient visual or motor deficits, have been described in aPL/APS patients; data come from isolated case reports and small series. However, the prevalence and potential association between aPL and MS-like syndrome have been poorly assessed. Studies from MS patients reported a prevalence of aPL of 6% for the IgG isotype and between 2% and 69% for the IgM isotype

[95–98], mostly in isolation and at low titers [99, 100]. Of five studies with healthy people or those with other neurologic diseases as a control group (96, 100–103), three reported an association between aCL and definite MS [96, 100, 103]. A higher frequency of atypical manifestations, such as headache or fewer oligoclonal bands in cerebrospinal fluid, occurred in patients with aPL [96, 104].

Myelitis is a rare clinical manifestation. The reported prevalence of myelitis in APS ranges between 0.4% and 4% [105, 106]. This manifestation is much more frequently seen in aPL-positive patients with SLE.

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

Multiple Sclerosis-like symptoms and transverse or longitudinal myelopathy occur rarely in SLE (1–2.5%, based on retrospective cohorts) [48, 107, 108]. Case reports of MS-like symptoms or myelitis have been more often described in aPL-positive SLE patients compared to aPL-negative SLE patients. However, due to the uncontrolled nature of these reports, there is no strong evidence for any association.

A recent retrospective study including 1193 SLE patients reported 14 cases of myelopathy, mostly longitudinal myelitis. Among these patients, 5/14 had aPL and two had active SLE disease. The authors conclude that myelitis is not associated with lupus disease activity (SLEDAI), but aPL were frequently detected in SLE patients with myelitis, compared to those without myelitis [108]. Data from a recent SLE patient survey showed that MS was considered in 29% of aPL-positive cases compared to 8% aPL-negative cases, demonstrating that in SLE patients aPL is associated with symptoms evocative of MS [109]. Two other studies did not confirm these associations [48, 90].

Neuroimaging Findings

Spinal cord MRI mostly displays longitudinal hyperintense lesions in T2 images, predominantly at the thoracic level. Enhancement after contrast injection is less common and imaging may be normal, especially in the early stages [108, 110]. Prognostic value of MRI abnormalities is unknown; the role of imaging in follow-up needs further assessment.

Pathophysiology

The pathophysiology of spinal cord injury/demyelination in aPL-related myelopathy or MS-like illness is unknown. Both ischemic and immunologic mechanisms have been suggested by imaging findings and clinical response of patients treated by immunosuppressive drugs and anticoagulant therapy [111].

Movement Disorders

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

Among movement disorders reported in aPL/APS patients, chorea, although rare, is the most frequently described. Data are limited to isolated case reports and one retrospective series of 14 patients. No specific feature of chorea (hemichorea, alternative hemichorea or generalized choreic movements) has been described. Chorea was mostly present at APS diagnosis. In APS, the reported prevalence of chorea is 1.3–4.5% in APS patients [25, 112]. Dystonia, ballismus, dyskinesia, Parkinson syndrome, and cerebellar ataxia have also been described in few cases [113–116].

Based on those studies, the quality of evidence was strong enough to conclude by the 14th International Congress on aPL Task Force that chorea is associated with aPL [65].

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

A retrospective study of 32 SLE patients with chorea found that 29/32 (90%) had persistent aPL according to the current APS Classification Criteria. Among them, 27 had LA, 19 aCL, and 11 aβ_2GPI, and 11 were triple positive, suggesting a close relationship between chorea and aPL in SLE [117]. Other controlled studies did not support this association [48, 90, 118]. In a prospective cohort of 374 APS patients, Stojanovich et al. reported chorea in 8% of aPL-positive SLE patients with no cases in aPL positive without SLE, suggesting that chorea was more related to the presence of SLE and aPL than the presence of aPL alone [67].

Pathophysiology

No animal model has assessed the role of aPL in chorea or other movement disorders. Occlusion of lenticulostriate arteries, producing ischemia of basal ganglia, is suspected in APS. However, neuroimaging findings have rarely confirmed these lesions. Some patients display improvement if treated with immunosuppressive drugs [117, 119]. Direct neurotoxic effects of aPL may be involved, and imaging studies suggest that inflammatory lesions are found in patients with chorea. A positron emission tomography (PET) scan studies report increased metabolic activity of basal ganglia [120].

Psychiatric Symptoms

Antiphospholipid Antibody-Positive Patients Without Lupus

Epidemiological and Clinical Findings

The relationship between psychosis, mania, depression, or bipolar disorders and aPL has been investigated in few studies and rarely described in APS patients [121, 122]. In one early study aCL were found in 24% of psychosis in-patients compared to 0% in healthy controls [123]. In a more recent study by Sokol et al. aCL were detected in 4/100 patients with psychosis, a prevalence similar to that of the general population [124]. When non-criteria aPL (antiphosphatidylethanolamine, antiphosphatidylserine, antiphosphatidylcholine) were tested, aPL were detected in 25% of patients. Anticardiolipin antibodies and LA have been detected in schizophrenic and untreated psychotic patients [125–127]. A retrospective cohort of 102 APS patients reported depression in 11 patients [105]. The findings have to be considered carefully, given discrepancies in methods for aPL assays, the lack of control at 12 weeks apart, and isolated low titers. Based on the results, it is difficult to conclude any association between psychosis or depression and aPL.

Antiphospholipid Antibody-Positive Patients with Lupus

Epidemiological and Clinical Findings

Psychiatric symptoms are well-known manifestations of neuropsychiatric SLE. The role of aPL in psychiatric manifestations of SLE is poorly investigated and depends highly on the definitions used for each psychiatric symptom. Their prevalence in aPL-positive SLE patients ranges from 3.5% to 27% [40, 48, 107, 128]. Previous studies have shown controversial findings on their association with aPL, with positive [129] and negative reports [77, 90]. To date, there is no strong evidence of an increased risk of psychiatric complications in the presence of aPL.

Pathophysiology

No studies have assessed the pathophysiological mechanisms of psychiatric manifestations in APS.

Other Manifestations

Idiopathic intracranial hypertension, transient global amnesia, and hypertrophic pachymeningitis have been reported in isolated cases of APS [130, 131]; whether these abnormalities are associated with aPL itself is still unproven.

Remaining Questions

Causal Link Between Antiphospholipid Antibodies and Neuropsychiatric Manifestations

Cognitive dysfunction and dementia are the most documented neuropsychiatric manifestations of aPL/APS. However, major methodological bias limits the interpretation of the results or the establishment of a potential mechanism. Standardization of the methods for measuring cognitive function in aPL/APS patients, and a recommended battery of tests, are needed. Larger prospective studies, which separate aPL carriers from APS and SLE-associated APS patients, with appropriate control groups, are necessary to determine if specific cognitive profiles are associated with aPL and/or APS. The relationship between stroke and cognitive impairment has not been properly assessed, and the biologic effects of aPL on the brain are not understood. While many reports show little or mild cognitive dysfunction in association with aPL, the impact on daily life has not been properly assessed.

Evaluation of cognitive dysfunction requires a long battery of tests that are impractical in routine clinical settings. Results obtained from brief cognitive screening, such as the Mini–Mental State Examination (MMSE), may be insensitive; this test's application and usefulness need to be further validated. No strong evidence of association of aPL with migraine is available. Seizures may likely be associated with aPL, but potential confounding factors such as stroke need to be considered to confirm aPL as an independent risk factor.

Neuroimaging

A larger use of neuroimaging in studies on neuropsychiatric manifestations associated with aPL is recommended to better evaluate the non-criteria manifestations that are independent to brain infarction and to better assess the clinical relevance of WMHL.

Table 10.1 Neurologic manifestations in antiphospholipid antibody-positive patients

Manifestations	Strength of evidence
Stroke/transient ischemic attack	Strong association based on large sample size prospective studies
Cognitive dysfunction Migraine Seizure/epilepsy	Frequently reported but based on small sample size prospective studies and retrospective cohorts
Psychosis Chorea Multiple sclerosis-like symptoms Myelitis Idiopathic intracranial hypertension Transient global amnesia Hypertrophic pachymeningitis	Infrequently reported based on small sample size prospective or retrospective studies or case reports

Pathophysiology

While the hypothesis of a direct immune effect of aPL on the brain (in addition to thrombosis) seems to be valid, specific interactions and the molecular mechanisms remain unknown.

Future Research Directions and Group Conclusions

The 14th International APS Congress called for new classification criteria, opening a discussion about the place of non-stroke neurological manifestations in future criteria. Future clinical research directions should better evaluate the association of each non-stroke neurological manifestation with aPL/APS (Table 10.1), with or without SLE, by conducting large multicenter studies that include well-defined patients, use strict definitions for each manifestation, and apply standardized aPL testing.

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Part IV
Therapeutic Aspects of Antiphospholipid Syndrome

Chapter 11
Prevention and Treatment of Thrombotic Antiphospholipid Syndrome

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Introduction

Antiphospholipid syndrome (APS) is a clinical syndrome characterized by both clinical and laboratory manifestations. Clinical manifestations most commonly include macro- and/or microvascular thrombosis, which may occur in the arterial or venous circulation, pregnancy morbidity, and thrombocytopenia. Laboratory criteria require persistent antiphospholipid antibody (aPL) positivity over a 12-week period for the lupus anticoagulant (LA), and/or anticardiolipin antibody (aCL), and/or anti-β_2-glycoprotein-I antibody (aβ_2GPI) at moderate-to-high titers. This chapter will cover the prevention and treatment of thrombotic manifestations of APS; therapies for the non-thrombotic manifestations of APS, pregnancy morbidity, and the special clinical circumstance of catastrophic APS are found in other chapters.

The prevention and treatment of thrombotic complications is focused on the use of anticoagulants and/or antiplatelet agents rather than other therapies such as immunomodulatory interventions. Antithrombotic therapy has been revolutionized in the last decade by the introduction of a variety of novel parenteral and oral anticoagulants. Despite ongoing studies, high-quality evidence for the efficacy and safety of many of these medications in patients with APS is still lacking, and in most cases older, but better studied, regimens remain the mainstay of anticoagulant therapy for patients with APS.

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Importance of Cardiovascular Disease and Venous Thrombosis Prevention in Antiphospholipid Antibody-Positive Patients

Risk modification strategies should always be considered for patients perceived to be at risk of atherosclerotic cardiovascular disease.

It has been noted in a number of studies that atherosclerotic cardiovascular events including stroke and myocardial infarction (MI) are increased in the presence of aPL. A case-control study nested within the Helsinki Heart Study noted higher titers of aCL in men who developed myocardial infarction (MI) or cardiac death, and men with MI/cardiac death had higher odds of having aCL titers in the upper quartile [1]. Similarly, Urbanus et al. [2] noted that young women with stroke or MI were more likely to have had LA/aβ_2GPI. In both these studies, additional risk factors such as smoking (1,2), anti-oxidized LDL antibodies [1], or use of the oral contraceptive pill [2] further increased the odds ratio associated with MI or stroke.

There are also increasing data that aPL may play a key role in the atherogenic process. Previous work from Hasunuma et al. demonstrated increased uptake of oxidized LDL by macrophage Fc-gamma receptors in the presence of aβ_2GPI [3]. In addition, endothelial cell apoptosis and aβ_2GPI may upregulate dendritic cells to drive inflammatory and oxidative stress responses, both of which may further increase endothelial dysfunction and damage [4]. Recent studies of monocyte gene expression profiling and miRNA analyses also have shown significant overlap between inflammation, oxidative stress, and atherogenic pathways in patients with primary APS, systemic lupus erythematosus (SLE), and SLE-associated APS [5, 6]. In the context of SLE, aPL are associated with subclinical atherosclerosis [7, 8] and clinical cardiovascular disease (CVD) events [9]. It remains a matter of debate however to what extent these data from a high-risk condition, SLE, are generalizable to primary APS. Andrade et al. found no difference in aortic pulse wave velocity (PWV) or carotid intima-media thickness (IMT) in primary APS vs healthy controls. However a subset analysis did show higher aortic PWV in primary APS patients with index arterial vs venous events [10].

To date there have been no formal studies attempting to determine how best to translate these observations into formal guidelines for cardiovascular disease (CVD) risk prevention. In a number of other inflammatory rheumatologic diseases, adjustment of population risk assessments has been recommended. For example, in rheumatoid arthritis (RA), European League Against Rheumatism (EULAR) recommends that all patients should be screened and their CVD risk calculated according to national CVD risk protocols [11]. The percentage 10-year risk calculated should then be adjusted by ×1.5 to adjust for the risk associated with RA if 2/3 of the following are present: RA for >10 years, anti-CCP antibodies, or rheumatoid factor. In SLE the excess risk is much higher and less consistent between studies; therefore, such a simple multiplication is inappropriate and likely to be highly inaccurate. The American Heart Association recommends SLE be considered as an additional risk factor in women, thus lowering the threshold and ideal targets for risk factors like

lipids and blood pressure [12]. Wajed et al. proposed that SLE be considered a "coronary heart disease equivalent" and therefore to adopt more stringent targets for other CVD risk factors [13]. In a recent study investigating the outcomes of SLE patients (with/without aPL) who participated in a 3-year free-of-charge CVD prevention counseling program, investigators demonstrated selected CVD risk factors that can be modified with continuous counseling [14].

Which, if any, of the above is most appropriate for patients with aPL is unclear. Many uncertainties remain, e.g., how to deal with single- vs triple-positive patients without previous thrombosis or obstetric complications, also whether any risk adjustment should be different for venous, obstetric, or arterial APS patients. It is likely that different targets, or risk score adjustments, will be needed for these different situations. However, in the absence of definitive studies, it is hard to draw firm conclusions.

After a review of the evidence to date, we recommend the following:

- All patients with aPL (with/without APS) should engage in regular cardiovascular screening programs according to national guidelines. Blood pressure control, smoking cessation, cholesterol and triglyceride management, and optimal diabetic control will help reduce the risk of arterial thromboembolism.
- Any such patient with an inflammatory rheumatic disease should be managed to minimize inflammatory disease activity, so-called treat-to-target (T-2-T) approaches. Hydroxychloroquine is an additional therapy that may facilitate achieving T-2-T targets and may have additional CVD protective properties (see below).
- Aspirin (see below) is frequently added, particularly to patients with "traditional" atherosclerotic risk factors and/or demonstrated atherosclerotic lesions (further discussed below).
- While a lower threshold for instituting additional CVD risk interventions seems reasonable, currently there is no consensus to support a particular threshold or risk adjustment.

Patients also should be counseled for other traditional thrombosis risk factors, such as oral contraceptive use, prolonged immobilization during a long flight, postmenopausal hormone therapy, pregnancy and postpartum period, and prophylaxis during surgical procedures. When aPL-positive patients undergo surgery, the most effective pharmacologic methods should be combined with physical anti-thrombosis methods such as intermittent venous compression [15].

Primary Thrombosis Prevention

Patients with aPL appear to be at increased risk of thrombosis irrespective of the patient's personal history of thromboembolism. In some studies, the risk of thrombosis in patients with positive aPL has been confounded by a high prevalence of SLE, which is in itself associated with thromboembolic complications [16]. However,

a recent meta-analysis of thrombosis risk associated with aPL positivity in patients without SLE demonstrated that such patients also have significant increases in the risk of thrombosis compared to the general population [17]. Reynaud and colleagues examined 30 studies enrolling a total of 16,441 patients and found the odds ratio (OR) for venous and arterial thrombosis to be 6.14 and 3.58, respectively, in patients with a positive LA test. Anticardiolipin antibodies were associated with ORs of 1.46 and 2.65 for venous and arterial thrombosis, respectively, and aβ_2GPI were associated with ORs of 3.12 for arterial thrombosis. The authors noted an overall low quality of evidence suggesting that the risk may have been inflated through reporting biases (i.e., the potential for event rates to have been inflated by reporting of patients with a history of the aPL and thrombosis, with a reduced likelihood of reporting of patients without these outcomes) [17]. The presence of more than one aPL probably increases the risk of thrombosis further. Pengo and colleagues examined 618 consecutive patients (of whom 55% had a prior history of thromboembolism) and compared patients with a history of thrombosis with those without [18]. They found that "triple positivity" (persistent positivity of LA, aCL, and aβ_2GPI) was associated with an odds ratio of 33 for thrombosis (95% confidence interval 7.0–157.6). However, both male gender and venous thrombosis risk factors were additionally, and independently, associated with thrombosis supporting the multifactorial nature of thrombosis in aPL-positive patients. The recently developed Global APS Score (GAPSS) score may help estimate the risk of future thrombosis for individuals with positive aPL [19, 20] (discussed in Chap. 9).

The observation that patients with aPL are at increased risk of first thrombosis, especially in the setting of other risk factors, would suggest that primary prophylaxis (i.e., the administration of prophylactic treatment prior to a first episode of venous or arterial thromboembolism) may be of benefit. However any benefit of primary thromboprophylaxis in aPL-positive patients must be weighed against the risk of bleeding associated with anticoagulants and/or antiplatelet agents and be evaluated in light of the likelihood of reductions in the risk of thrombosis due to modification of other risk factors.

Aspirin is an optional preventative therapy in a variety of patients at risk of both arterial and venous thrombosis. Arnaud and colleagues examined ten observational studies and one randomized control trial including 1208 patients with positive aPL who experienced a total of 139 venous and arterial thromboembolic events. Aspirin-treated patients were protected against a first arterial event (OR 0.48 (0.28–0.82)) but not against a first venous event (0.58 (0.32–1.06)). Interpretation on this analysis is limited by heterogeneity, inclusion of both observational and interventional studies, and a lack of consistency in laboratory criteria for diagnosis. The finding that the beneficial effect of aspirin was confined to the non-prospective studies highlights the uncertainty about how (or whether) these observations should impact clinical practice [21]. Similar findings were reported in a more recent patient-level analysis [22].

The lack of convincing evidence of benefit of aspirin suggests that more intensive anticoagulant options should be undertaken with great care and only in highly selected patients. For most patients with aPL, it is likely that the bleeding risk associated with anticoagulant use would outweigh the small potential absolute benefit of

primary thrombosis prevention. Cuadrado et al. examined the number of thrombotic events among patients with aPL and a history of SLE and/or prior obstetric morbidity randomized to receive low-dose aspirin or low-dose aspirin plus warfarin targeted to an international normalized ratio of 1.5. A total of 82 patients were allocated to low-dose aspirin and 84 to low-dose aspirin and warfarin. Over the total enrolment period of 5 years, eight patients had a thrombotic event (four per arm, p = NS). Eleven patients allocated to dual therapy reported abnormal bleeding compared with none allocated to low-dose aspirin. The authors concluded that low-dose aspirin plus warfarin cannot be justified as a primary prevention strategy given the lack of evidence of efficacy and reasonable evidence of toxicity [23].

Hydroxychloroquine has multiple beneficial effects in SLE patients, and it may reduce the levels and/or activity of aPL [24]. A prospective study of hydroxychloroquine in patients with aPL was terminated early due to a low recruitment rate exacerbated by a prolonged manufacturing shortage and price increase of hydroxychloroquine in the United States [25]. A recent systematic review found no evidence of a therapeutic benefit for hydroxychloroquine in patients with aPL concurrently treated with aspirin [22]. Toxicities include ocular abnormalities which increase toward 1% after 5–7 years of use or a cumulative dose of 1000 g, mandating regular ophthalmologic examinations [26].

Case reports and other very low-quality evidence have suggested that a variety of interventions, including intravenous immunoglobulin and immunosuppression, may be of benefit in selected patients with aPL; however, such evidence is highly prone to bias and is not relevant to the "average patient" with aPL and no prior history of thrombosis.

In summary, patients with aPL (particularly those with other systemic autoimmune diseases and multiple serologic abnormalities) are at an enhanced risk of both venous and arterial thrombosis compared with patients in the general population. Aspirin can be considered to reduce the risk of a first thrombotic event in patients with persistently positive aPL, especially in those with other cardiovascular risk factors; however, the net benefit of this intervention remains uncertain. More intensive prophylactic treatment, such as a combination of aspirin with low-intensity warfarin, appears ineffective and is associated with enhanced toxicity. Hydroxychloroquine should be used as part of a strategy to mitigate SLE complications, but the effectiveness is unknown in aPL-positive patients without other systemic autoimmune diseases. There is insufficient evidence to justify other prophylactic treatment strategies against thrombosis in patients with aPL, with or without underlying autoimmune diseases. Risk factor modification should be undertaken in all patients to reduce their risk of atherosclerotic vascular disease and venous thromboembolism.

Secondary Thrombosis Prevention

In general, secondary prevention of thromboembolism involves the administration of anticoagulation as well as the identification and management of modifiable risk factors as discussed above.

The risk of recurrent thrombosis in patients with aPL has not been clearly identified. In general, patients are divided into those with one or more venous thromboembolic events (without arterial events) or those with prior arterial events (irrespective of their history of venous events). Garcia et al. highlighted the poor quality of data describing the risk of recurrence in patients with aPL and prior venous thromboembolism. In their study of more than 500 patients with aPL (compared with more than 1900 patients without aPL), the unadjusted risk for recurrent venous thromboembolism after stopping anticoagulation was 1.53 (0.76–3.11) for patients with aCL and 2.83 (0.83–9.64) for patients with a positive LA test. Neither reached statistical significance, and the authors concluded that positive aPL tests increase the risk of recurrent venous thromboembolism; however, "the strength of this association is uncertain because the available evidence is of very low quality" [27].

Venous Thrombosis (Without a History of Arterial Thrombosis)

Patients with persistent positivity of aPL and a history of one or more venous thrombotic events are adequately treated with warfarin administered to achieve an international normalized ratio (INR) of 2.0–3.0. This conclusion is based upon two randomized controlled trials both of which were designed to demonstrate superiority of higher-intensity warfarin and both of which counterintuitively showed a non-significantly increased risk of recurrent thrombosis when high-intensity warfarin (target INR > 3.0) was compared to a more conventional approach (INR of 2.0–3.0) [28, 29]. No studies have examined the optimal duration of anticoagulation in patients with persistent positive aPL and one or more venous thromboembolic events; in the absence of such evidence, most "experts" support extended duration therapy for such patients [30]. There is no evidence to support the addition of aspirin to warfarin administered to achieve an INR of 2.0–3.0 in patients with a history of aPL and prior venous thromboembolism. Although the direct oral anticoagulants are appealing alternatives to warfarin in many clinical settings, there are only sporadic reports on their use in patients with aPL [31, 32]. The results of randomized controlled trials on their efficacy and safety in patients with APS are pending (discussed in Chap. 18).

Other agents, such as intravenous immunoglobulin or immunosuppression (e.g., with corticosteroids), are the subject of anecdotal reports of success in preventing recurrent venous thromboembolism; however, such reports should be regarded with skepticism, given small numbers, short follow-up periods, and exceptional case selection. Agents such as therapeutic dose low-molecular-weight heparin should be reserved for patients with recurrences despite usual therapeutic anticoagulation [33].

In summary, patients with aPL and a history of prior venous thrombosis and no previous arterial thrombosis can be adequately treated with warfarin with a target INR of 2.0–3.0. The direct oral anticoagulants are an appealing option for such

patients; however, until reasonable evidence on their safety and efficacy is available, their use should be confined to selected patients who are fully informed with respect to the lack of good-quality evidence for their use.

Arterial Thrombosis (Irrespective of the History of Venous Thrombosis)

Patients with persistent aPL and a history of arterial thrombosis represent a more difficult clinical scenario. As previously discussed, risk factor modification is likely to be critical, particularly in older patients or those with additional risk factors.

The "aPL and Stroke Study" enrolled a selected group of patients who had a single positive aPL determination and a prior stroke, treated with aspirin or warfarin (administered to a nonstandard therapeutic intensity of 1.4–2.8), and followed for recurrent vascular events over a 2-year period. The rates of recurrent vascular events were similar in both groups. Criticisms of this study include the failure to enroll patients with persistent antibody positivity, the use of nonstandard warfarin intensity, and a demographic profile quite different from that generally seen in patients acknowledged to have APS [34]. A recently published follow-up analysis wherein selected patients underwent serial testing for aCL, $a\beta_2$GPI, and anti-phosphatidylserine antibodies from stored serum found that none of persistently present aCL and anti-phosphatidylserine antibodies or transiently positive $a\beta_2$GPI, anti-phosphatidylserine antibodies, or aCL were associated with an increased risk of recurrent thrombosis. Persistently positive $a\beta_2$GPI were associated with reduced time to event/death (hazard ratio 2.86 (1.21–6.76)). Unfortunately, LA, the antiphospholipid antibody test most strongly related to thrombosis, could not be determined [35].

The two randomized controlled trials which have established warfarin administered to a target INR 2.0–3.0 as the preferred treatment for patients with prior venous thromboembolism and persistent aPL positivity enrolled a relatively small number of patients with prior arterial thrombosis [28, 29]. As a result, there are limited data to support any particular treatment strategy in these patients. Furthermore, given the low rate of recurrent thrombosis observed to date in studies of anticoagulant strategies in such patients, it is unlikely that further methodologically rigorous evidence will become available – the required sample sizes for definitive studies are likely to make such studies unfeasible. Potential strategies include "usual-intensity warfarin" (target INR 2.0–3.0), with or without aspirin, higher-intensity warfarin (target INR greater than 3.0) with or without aspirin, alternate anticoagulant strategies (e.g., long-term therapeutic dose low-molecular-weight heparin), or the combination of one of these anticoagulant strategies with an alternate treatment designed to reduce the likelihood of recurrence [30].

"Usual-intensity warfarin" is the preferred treatment for the prevention of recurrent arterial thrombosis in many clinical centers and has been recommended by widely regarded, evidence-based guidelines [36]. The addition of aspirin is fre-

quently considered, particularly in patients with additional atherosclerotic vascular risk factors who would be treated with antiplatelet therapy if they did not have persistent aPL positivity. As noted, the safety of this treatment remains controversial given the lack of good-quality, prospective data.

"Higher-intensity warfarin" is a preferred option in some clinical centers. However, maintaining an INR above 3.0 is technically difficult and may increase the risk of bleeding. Such therapy should be confined to specialized institutions familiar with the risks and benefits of more intense warfarin therapy. Addition of aspirin to "higher-intensity warfarin" is likely to increase the risk of bleeding to an unreasonable degree in many patients; as a result, the combination of aspirin plus higher-intensity warfarin should be confined to those patients for whom other, less hazardous, antithrombotic strategies would be expected to fail.

Alternate therapeutic strategies, such as long-term therapeutic dose low-molecular-weight heparin, should be confined to patients with objectively confirmed failure of therapeutic anticoagulation with warfarin. The cost, complexity and potential for bleeding with such therapy make it an unreasonable "first choice" for such patients.

Certain patients with APS present with a fulminant "catastrophic" thrombotic course characterized by recurrent arterial and/or venous thrombosis despite adequate anticoagulation. Such patients may be treated with intensified low-molecular-weight heparin or more aggressive therapies such as immunomodulation with immunosuppressive drugs, intravenous immunoglobulin, plasma exchange or rituximab (discussed in a Chap. 17).

In summary, there is a lack of good-quality evidence to support any particular therapeutic strategy in patients with persistent aPL positivity and a history of arterial thrombosis (with or without prior venous thrombosis). Standard-intensity warfarin (administered to an INR of 2.0–3.0) is used in many centers based on limited data from two randomized controlled trials. Some centers may choose to use higher-intensity warfarin (administered to an INR of greater than 3.0). Aspirin is frequently added, particularly to patients with "traditional" atherosclerotic risk factors and/or with demonstrated atherosclerotic lesions with concomitant usual intensity warfarin; however, evidence for additional efficacy over and above that of warfarin is limited, and the addition of aspirin will increase the risk of bleeding. Aspirin alone may be the preferred agent in some patients presenting with stroke (e.g., perhaps those with low-risk serological characteristics). The direct oral anticoagulants are not indicated for the prevention of recurrent arterial thrombosis in most jurisdictions and as such should be avoided in these patients. Other agents, such as therapeutic dose low-molecular-weight heparin, may be considered in patients with objectively confirmed recurrent thrombosis despite adequate warfarin therapy. An additional role of hydroxychloroquine in the secondary prevention of arterial thrombosis is suggested; however, no studies have yet demonstrated such an effect, their use being thus restricted to empirical therapy for patients with recurrent thrombosis despite correct antithrombotic treatment or with bleeding complications or risk that preclude the use of anticoagulant drugs. Further research is required to better define "optimal therapy" for these patients, given the small number of patients currently enrolled in studies and the catastrophic negative outcomes of recurrent thrombosis.

Group Conclusion (Table 11.1)

Prevention of first and recurrent thrombosis in patients with aPL is a high clinical priority since such patients appear to be at increased risk of thrombosis. Primary prevention with aspirin is considered in patients with persistently positive aPL, especially in those with additional indications for aspirin, but should not be considered "standard" in patients who do not have such risk factors. Secondary prevention of patients with venous thrombosis should consist of warfarin administered to an INR of 2.0–3.0. Most patients with prior arterial thrombosis are treated with warfarin at a target INR of 2.0–3.0; however, evidence to support this intervention is weak. In some centers, there is a preference for higher-intensity warfarin or addition of low-dose aspirin to a standard intensity anticoagulation regime. The published experience with direct oral anticoagulants to treat thrombosis in patients with aPL is very limited. Good-quality studies are urgently needed for many clinical decisions relevant to patients with aPL who have, or at risk of, thrombosis as current evidence to guide practice is limited.

Table 11.1 Group recommendations for the prevention and treatment of thrombotic antiphospholipid syndrome

Cardiovascular disease and venous thrombosis prevention
Screening for and aggressive management of conventional atherosclerosis risk factors
Screening for and elimination of venous thrombosis risk factors
Patient education
Primary thrombosis prevention
Low-dose aspirin in persistently moderate-to-high titer aPL-positive patients who have additional cardiovascular risk factors. No evidence that aspirin benefits the patients who do not have additional cardiovascular risk factors
No anticoagulation except if indicated for other conditions
Secondary thrombosis prevention
Warfarin with a target international normalized ratio (INR) of 2.0–3.0 for venous thrombosis
Warfarin with a target INR of 2.0–3.0 with consideration of low-dose aspirin for arterial thrombosis (the group acknowledges the fact that given the lack of strong data on arterial thrombosis, some centers prefer warfarin with a target INR of 3.0–4.0)
No use of direct oral anticoagulants until the results of the ongoing randomized clinical trials are available
Indefinite anticoagulation in aPL-positive patients with unprovoked thrombosis with continuous assessment of the bleeding risk.
Optimal therapy of patients with provoked venous thrombosis is unknown. Therapy for a minimum of 3 months and until the provoking risk factor is eliminated should be provided to all patients. Strong consideration of extended duration anticoagulation recommended for most patients except perhaps those identified to have a high risk of bleeding.

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Chapter 12
Prevention and Treatment of Obstetric Antiphospholipid Syndrome

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Introduction

Initial excitement surrounding the treatment of antiphospholipid syndrome (APS) to improve pregnancy outcomes, particularly to avoid pregnancy loss, was understandable. In the mid-1980s there was no proven "treatment" for recurrent miscarriage or otherwise unexplained fetal death. Early case series included patients with lupus anticoagulant (LA) and involved treatment during pregnancy with glucocorticoids and low-dose aspirin (LDA). Heparin was used in hopes of improving placental blood flow. Both prednisone and heparin seemed to improve pregnancy outcomes in uncontrolled case series. A multicenter randomized trial found that heparin and LDA were as effective as glucocorticoid and LDA and were associated with a better safety/adverse event profile [1]. This trial, however, was limited in terms of small sample size and uncertainties regarding patient selection. By the early 1990s, the most commonly used treatment of pregnant patients with APS was established as heparin and LDA, and so it remains.

This chapter has two sections concerning pregnant aPL-positive patients: assessment and treatment. Treatment is discussed separately for two different populations: (a) those with recurrent early miscarriage; and (b) those with a history of thrombosis, mid-second or third trimester fetal death, or early delivery because of severe preeclampsia or placental insufficiency. (The term "obstetric APS" used in this text refers to both forms.)

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Assessment of Antiphospholipid Antibody-Positive Patients with Pregnancy Morbidity

Given the relative infrequency of fetal death (loss of a fetus ≥10 weeks of gestation) relative to early pregnancy loss (miscarriage, before 10 weeks), proper assessment should document the gestational age of the pregnancy death (not, as is commonly done, the gestational age at diagnosis, when clinical symptoms or serendipitous diagnosis occur). Thus, an embryo that dies at eight menstrual weeks of gestation, but is first diagnosed at 11 weeks, is often wrongly considered to be a fetal death [2].

Several objective criteria can be used to confirm gestational age of a loss. Crown-rump length measured on ultrasound ≥3.0 cm documents 10-week gestational age. Other methods include having heard fetal heart tones with a handheld Doppler device at a specified time or assessment of fetal size directly after delivery or uterine evacuation [2].

The fetal death should be unexplained to qualify as an APS criterion. Other known causes of pregnancy loss should be excluded. This is not always done for a variety of reasons, including an assumption that an evaluation "will not bring my baby back", fears or misconceptions about testing, avoidance of uncomfortable or unpleasant issues by the clinician, and financial concerns.

Considering early spontaneous abortion, chromosomal abnormality is the most frequent reason in patients with two or more miscarriages, a frequency similar to that found in patients with a single sporadic spontaneous abortion [3]. Investigation of chromosomal abnormalities in abortions is infrequently performed in clinical practice because of cost, accessibility of the test, and technical limitations. Another genetic cause of recurrent miscarriage is parental balanced translocations, detectable through analysis of parental karyotype [4].

Uterine malformations occur in up to 15% of women with recurrent pregnancy losses. Evaluation with three-dimensional ultrasound, hysterosalpingogram, diagnostic hysteroscopy, and/or magnetic resonance imaging (MRI) may be considered if suspected. Although more related to second trimester losses, some authors advise that uterine cavity evaluation should be part of an evaluation. Uncontrolled endocrine abnormalities, such as diabetes and hypothyroidism, may also cause early abortion; treatment can improve gestational outcome [4].

Recent well-designed studies have questioned whether inherited thrombophilias, infections, and alloimmune factors, previously associated with recurrent miscarriages, are worth investigating. The current answer is no, because of lack of established association and/or effective treatment [4].

The optimal evaluation for potential causes of fetal death is uncertain. Considering cost, inconvenience, and potential yield, it is reasonable to focus on tests that have a high chance of providing useful information, on the most frequent conditions and on those, such as APS, with implications for subsequent pregnancies. Although many tests are recommended in the evaluation of stillbirth [5], the most consistently useful tests are perinatal autopsy, placental histology, and genetic testing (either karyotype or chromosomal microarray). Testing for aPL is helpful in cases in which

fetal growth restriction, placental insufficiency, or severe preeclampsia has occurred. Other testing is best limited to cases in which clues from the clinical history or results of autopsy or other findings suggest specific diagnoses.

Determining a cause of death can be difficult, even after a complete diagnostic evaluation. There are many different causes of fetal death, and there may be uncertainties regarding a cause. There may be more than one cause; many potential etiologies may be risk factors rather than causes, since they often occur in live births, and some conditions, for instance, diabetes, may contribute to fetal death when severe but not when mild.

Over 60 classification systems have been developed to catalog potential causes of stillbirth. None is uniformly accepted worldwide. A major hurdle is a lack of a gold standard to validate the classification system. The Stillbirth Collaborative Research Network (SCRN), using rigorous definitions and the best available evidence to create a classification system named INCODE [6], conducted a multicenter case-control study of stillbirths and live births in the USA in five regions. In a cohort of over 500 stillbirths, INCODE identified a probable cause of death in 60.9% and a possible or probable cause in 76.2% [7].

The results of testing for antibodies to cardiolipin (aCL) and to β_2GPI-glycoprotein-I (aβ_2GPI) in the SCRN study underscore the importance of a thorough evaluation for fetal death. A higher proportion of women with stillbirth (excluding fetal anomalies) had positive tests for IgG aCL than did controls (5.0% vs 1.0%; OR 5.30; 95% CI 2.29–11.76) [8]. IgM aCL and IgG aβ_2GPI were also associated with stillbirth. Although 56 women had positive tests for aPL, only 14% of these had APS as a probable cause based on INCODE [8]. Several cases with aPL had major genetic abnormalities.

The number of requirements for valid evaluation of pregnancy loss highlights a glaring oversight of many published trials: failure to meticulously characterize [2] and stratify subjects according to the gestational age of prior pregnancy losses, exclusion of other causes, and number of prior losses required (or allowed) for inclusion in the trial. Studies are difficult to compare because the definition of aPL positivity varies [9, 10], many reported subjects having indeterminate or low levels. In the majority of published trials, many subjects meet neither the 1999 [11] nor the 2006 [12] criteria for APS. Most published trials are plagued by small sample size, lack of blinding, and lack of a placebo control.

The associations between aPL and preeclampsia and placental insufficiency are addressed in Chap. 6. Two systematic reviews emphasize that the association between aPL and preeclampsia rests on the link to preeclampsia with severe features, typically in women who are delivered preterm [13, 14].

Although the review by do Prado et al. included 12 primary studies in a meta-analysis, confirming the strong association of aPL with severe preeclampsia (OR 11.15, 95% CI 2.66–46.75), only half of the studies specified criteria for severe preeclampsia.

The review by Abou-Nassar et al. included 28 studies. LA positivity was associated with preeclampsia in case-control (OR 2.34, 95% CI 1.18–4.64) but not in cohort studies (OR 5.17, 95% CI 0.60–44.56) [14]; aCL was associated with preeclampsia

in case-control studies (OR 1.52, 95% CI 1.05–2.20) but not in cohort studies (OR 1.78, 95% CI 0.39–8.16); and, paradoxically, aβ_2GPI was associated with preeclampsia in cohort studies (OR 19.14, 95% CI 6.34–57.77) but not in case-control studies. This review also evaluated preeclampsia and placental insufficiency, defined by late fetal loss, intrauterine growth restriction (IUGR), or placental abruption and found an association of IUGR with LA in case-control studies (OR 4.65, 95% CI 1.18–4.64) and with aβ_2GPI in cohort studies (OR 20.03, 95% CI 4.59–87.43) [14].

Both systematic reviews note that existing studies have high variability in their definitions of preeclampsia and placental insufficiency, often including women who deliver at term, and inclusion inconsistent with the revised Sapporo APS Classification Criteria [12]. Existing studies also include widely variable definitions of aPL positivity, including considering low thresholds of aPL, and not testing for all three aPL (LA, aCL, and aβ_2GPI). Only a handful of studies report confirmatory testing of aPL at 12 or more weeks after initial test.

An abstract presented by Gibbins and colleagues during the 15th International Congress on Antiphospholipid Antibodies used strict cutoffs for aPL positivity (>40 GPL or MPL), tested all three aPL (LA, aCL, and aβ_2GPI), and only considered a patient a case if she was delivered before 36 weeks. The investigators found an association between aPL and severe, preterm preeclampsia or placental insufficiency [15]. In this study, 10.5% of patients with preeclampsia or placental insufficiency tested positive for aPL, compared to 1.5% of controls (OR 7.59, 95% CI 1.63–35.42). Only 52.6% of women returned for repeat confirmatory testing.

Treatment of Antiphospholipid Antibody-Positive Patients with Pregnancy Morbidity

In many trials, it is difficult to determine what proportion of enrolled subjects had only pregnancy losses prior to 10 weeks of gestation versus fetal deaths at/beyond 10 weeks of gestation, a distinction required by the criteria for APS [12]. Subjects are more frequently diagnosed with APS because of recurrent early miscarriage than because of prior thrombosis, fetal death, or early delivery for severe preeclampsia or placental insufficiency. Among women with recurrent early miscarriage who also test positive for aPL, the frequency of history of thrombosis, stillbirth, or pregnancy complicated by severe preeclampsia is small. These characteristics, greater numbers, and lack of comorbid conditions make a population of recurrent early miscarriage patients more amenable to treatment trials. Also, women diagnosed with APS because of prior thrombosis, fetal death, or severe preeclampsia are difficult to enroll in trials that include a placebo arm [9]. Because women with prior pregnancy losses and women with preterm birth due to preeclampsia or placental insufficiency desperately seek a modifiable cause, heparin and LDA are commonly prescribed when a positive test for aPL is found, even though the patient does not meet clinical or laboratory criteria for APS.

Many experts note the marked trial heterogeneity caused by variable entry criteria [9, 16]. In one review of association between aPL and recurrent miscarriage, almost one third of 46 studies analyzed patients with first trimester losses together with patients with second and/or third trimester losses, and 15 studies did not mention the gestational age. This is problematic, as pathogenesis of abortion varies with duration of gestation and chromosomal abnormalities being more common in early losses [9].

Obstetric Antiphospholipid Syndrome Based on Recurrent Early Miscarriages

The most frequently quoted treatment trials of obstetric APS date from the 1990s and early 2000s. They included primarily women with recurrent early miscarriage. Most of these trials involved treatment with a heparin, prednisone, LDA or a combination of these agents. Only one trial [17] was blinded and placebo controlled. Only 40 patients completed the study, and there were no differences between patients who received LDA and those receiving placebo. The live birth was 80% or better in both groups; all but two infants were delivered at term. At least three other trials [18–20], all published before 2000, included no-treatment arms. The number of subjects in each of these trials was, and outcomes without treatment were, good. Silver et al. [21] randomized patients to prednisone plus LDA or LDA alone. Not only was there no difference between the groups in the rates of live births, but there were no perinatal losses at all. Importantly, women who received prednisone had more premature deliveries.

Four trials that included women with APS diagnosed primarily because of recurrent early miscarriage compared heparin plus LDA to LDA alone [22–25]. In two trials, the proportion of successful pregnancies were higher in the unfractionated heparin (UFH) arm [22, 23]. The other two trials used low-molecular-weight-heparin (LMWH) and found no benefit with the use of LMWH [24, 25]. The live birth rates in the LDA-only patients were 70% and 75%.

Two recent trials also compared either UFH or LMWH to LDA alone. In one trial, investigators randomized 72 women with predominantly recurrent early miscarriage who had > 18 GPL IgG aCL [26]. Those randomized to heparin plus LDA had a higher rate of live birth (84.8%) than did those in the LDA only group (61.5%). Another trial randomized 141 women with two or more miscarriages and who had LA, aCL IgG > 15 U, and/or IgM > 25 U on two occasions [27]. Women randomized to the bemiparin (a LMWH) did not take LDA. The live birth rates were 86% in the bemiparin group and 72% in the LDA group; the difference was significant.

Notably, only one trial [27] studied more than 100 patients. Given what is known about pregnancy outcomes in placebo- or aspirin-treated groups, some may reasonably conclude that statistical proof for or against treatment remains in question. The EAGeR trial [28], which randomized otherwise healthy women (who were not known to have aPL) with one or two prior miscarriages, to preconception LDA or placebo, was powered to find a 10% absolute difference in live birth rates and

enrolled 1278 subjects. The live birth rates were the same (82%) in each arm among women with pregnancy test and ultrasound-confirmed pregnancies.

Three other studies comparing UFH to LMWH (each paired with LDA) found no difference in pregnancy outcomes among women with predominantly recurrent early miscarriage [29–31]. Two trials of women with APS primarily diagnosed because of recurrent early miscarriage compared heparin plus LDA to intravenous immunoglobulin (IVIG) [32, 33]. In both, the live birth rate was over 70% in the heparin plus LDA group and under 60% in the IVIG group, results that weigh against the use of IVIG in these patients.

In summary, a critical review of existing trials that enrolled women with APS diagnosed predominantly because of recurrent early miscarriage allows several contrasting conclusions. Marked trial heterogeneity, largely because of variable patient entry criteria, makes it difficult to compare results. Other interpretation problems include the definition and gestational age of a confirmed pregnancy, small sample sizes, and lack of blinding. The successful pregnancy rates in no-treatment or LDA-treated patients varies from less than 50% to well over 70%.

One can conclude that the recommendation of heparin plus LDA to improve pregnancy outcomes in this subset of APS patients is based on evidence that is conflicting at best and unacceptably weak at worst. The most recent American College of Obstetricians and Gynecologist Practice Bulletin states that, for women with APS without a preceding thrombotic event, “expert consensus suggests that clinical surveillance or prophylactic heparin” may be used in the antepartum period and that “prophylactic doses of heparin and LDA during pregnancy and 6 weeks postpartum should be considered” [34].

Obstetric Antiphospholipid Syndrome Based on Fetal Loss or Early Delivery Due to Severe Preeclampsia or Placental Insufficiency

It is difficult to randomize women with obstetric APS to a placebo arm, especially those with history of fetal losses. Only a few trials state how many patients with APS have a history of mid-second or third trimester fetal death or early delivery because of severe preeclampsia or placental insufficiency, much less analyze them separately. Also, the role of fetal monitoring and its impact on perinatal outcome are sometimes poorly defined. Experts agree that women with a history of thrombosis cannot be randomized to a regimen without thromboprophylaxis.

Nonetheless, two trials deserve consideration. In a randomized trial [25] of heparin plus LDA versus LDA alone that included patients with both inherited thrombophilias and aPL, 25% of subjects had a history of fetal death after 14 weeks of gestation (women with prior thrombosis were excluded). Although there were no differences between treatment groups with regard to live births or fetal losses after 14 weeks, only half the subjects had aPL, and they were not separately analyzed. In

another small trial [35] that randomized women with APS to receive heparin plus LDA or IVIG plus heparin plus LDA, over 85% of subjects had a history of prior fetal death. All enrollees had live births, but the preterm birth rate was higher in the IVIG group.

Meaningful information regarding the impact of treatment on pregnancies of patients with aPL comes from the prospective, observational PROMISSE study. This multicenter effort enrolled pregnant women ≤18 weeks of gestation with aPL, APS, or systemic lupus erythematosus (SLE). Women with comorbid conditions that placed them at higher risk for adverse pregnancy outcomes, for instance, significant proteinuria, elevated serum creatinine >1.2 mg/dL, or blood pressure ≥140/90 at the time of enrollment, were excluded. Fetal monitoring was prescribed and followed by the investigation team. The patients' physicians made all treatment decisions; the great majority of those with aPL or APS were treated with a heparin. An initial analysis found that, despite nearly universal treatment with heparin, 64 women with repeatedly positive tests for LA had a 39% rate of fetal death, preterm delivery prior to 34 weeks because of gestational hypertension or placental insufficiency, small for gestational age infant, or neonatal death related to early delivery [36]. By comparison, women with aPL who were negative for LA had a rate of adverse pregnancy outcome less than 10%. Even among the 29 women with greater than 40 GPL units of aCL who were negative for LA, the rate of adverse pregnancy outcome was only 8%. Patients were more likely to have an adverse pregnancy outcome if they had a history of prior thrombosis or SLE. Not only do these findings raise questions about impact of heparin on adverse pregnancy outcomes, they emphasize the need for carefully stratifying patients when designing trials and analyzing treatment effects.

Ruffatti et al. [37] also emphasized the relationship between certain clinical characteristics and adverse pregnancy outcome despite antithrombotic treatment. In comparing clinical characteristics of successful versus unsuccessful APS pregnancies, the investigators found that more unsuccessful pregnancies occurred in women who were LA or "triple" positive and that unsuccessful pregnancies were associated with a history of prior thrombosis or SLE and with prior pregnancy morbidity. The investigators treated 18 pregnancies of 14 women (triple positive for aPL) with weekly plasmapheresis and fortnightly IVIG in addition to heparin plus LDA [38]. Seventeen of the pregnancies were successful, but preterm birth was common (mean gestational age of delivery 33 weeks), and one premature born infant succumbed to infection.

Two trials aimed to discern whether heparin would decrease the rate of adverse pregnancy outcomes *in women without APS*, but with prior hypertensive disorders or other consequences of abnormal placentation. The TIPPS trial [39] reported 284 women with histories of severe preeclampsia, small-for-gestational-age infants, or placental abruption who completed a trial that randomized patients to dalteparin in thromboprophylactic doses or no dalteparin. There were no differences between the two arms in terms of pregnancy loss or placenta-mediated complications. The FRUIT-RCT [40] reported 139 women with an inheritable thrombophilia and a history of delivery before 34 weeks for hypertensive disease or small-for-gestational-age infants who were randomized to receive either dalteparin in prophylactic doses

with LDA or LDA without dalteparin. No women in the dalteparin plus LDA arm had recurrent hypertensive disorders <34 weeks compared to 8.7% in the LDA arm, a statistically significant difference. There were no differences between treatment arms in patients with hypertensive disorders, irrespective of gestational age or fetal deaths. These trials do not settle the question as to whether or not women with APS adverse pregnancy outcomes should be treated with heparin plus LDA, but they were well-designed, multicenter efforts that should service models for future trials.

Aspirin is the only currently accepted treatment to prevent preeclampsia and placental insufficiency in obstetric APS. The US Preventive Services Task Force (USPSTF) recommends aspirin for preeclampsia prophylaxis in high-risk women, but this recommendation is not specific to women with APS [41, 42]. Antiphospholipid syndrome is listed in the USPSTF report, a risk factor that meets criteria for use of aspirin 81 mg/day after 12 weeks of gestation, and quotes a 24% risk reduction for preeclampsia, 14% for preterm birth, and 20% for IUGR [42].

Therapeutic agents that have been studied specifically for prevention and treatment of obstetric APS include IVIG, LMWH, and plasmapheresis. Agents showing potential promise include pravastatin and complement inhibition.

Intravenous immunoglobulin, 1 g/kg for two consecutive days per month starting at less than 12 weeks, was evaluated in a multicenter, randomized, placebo-controlled trial of women with known APS, all of whom received heparin and LDA. Outcomes were preeclampsia, fetal growth restriction, and placental insufficiency. Only 16 women were enrolled in this trial; the only difference between groups was an increased rate of preterm birth in the IVIG group (100% vs 33%, $p = 0.01$) [35].

Low-molecular-weight-heparin was recently evaluated by van Hoom and colleagues in a multicenter, randomized clinical trial of 32 women with aPL and a previous delivery for hypertensive disorder of pregnancy or a small for gestational age (SGA) neonate prior to 34 weeks. Antiphospholipid antibodies were considered positive if aCL was ≥10 GPL/MPL and/or LA was positive on at least two occasions. All women received daily LDA. The LMWH regimen chosen in this trial was dalteparin with weight-based dosing, initiated between 6 and 12 weeks, after ultrasound confirmation of ongoing intrauterine pregnancy. The primary outcome was recurrent hypertensive disease before 34 weeks. There was no difference between treatment arms in the primary outcome or secondary outcomes (small for gestational age, pregnancy loss, preterm birth, or side effects). Gestational age at delivery and birthweight did not differ between groups [43].

Plasmapheresis has been proposed to remove pathogenic antibodies from maternal circulation and potentially limit obstetric morbidity. One case series evaluated 18 women with obstetric APS. Women received prednisone 10 mg/day as soon as fetal cardiac activity was confirmed sonographically. They then began plasmapheresis, typically three times per week until 16–18 weeks, then less frequently, guided by laboratory parameters. All women had live births, of which four (22%) were preterm [44]. Absent formal clinical trials, it is impossible to draw definitive conclusions from this single case series.

Pravastatin, an inhibitor of cholesterol synthesis, is a therapeutic agent of interest in placental insufficiency and endothelial dysfunction because it appears to be effective in animal models of APS [45] and has now been studied in humans [46]. A retrospective trial included women meeting obstetric APS criteria, all of whom developed preeclampsia or IUGR, and all of whom received LMWH and LDA from confirmation of pregnancy. Ten women were maintained on LMWH and LDA alone after diagnosis of preeclampsia and/or IUGR, and 11 received pravastatin 20 mg as well at diagnosis. Pregnancy prolongation was 13 weeks in the pravastatin arm versus 4.5 weeks in the women who did not receive pravastatin, $p < 0.001$ [47]. No stillbirths occurred in the pravastatin cohort, whereas three occurred in the standard treatment cohort. Although this is not a randomized, clinical trial, it suggests that pravastatin deserves further study as a potential therapy for APS-related obstetric morbidity, even after diagnosis of preeclampsia or IUGR.

Salmon and colleagues showed that obstetric APS is associated with complement activation, which initiates and augments the pro-inflammatory, pro-adhesive, procoagulant milieu [48–52] and may injure placental angiogenesis and lead to placental insufficiency [52–54]. Thus agents that inhibit complement activity may limit the morbidity. Eculizumab, a targeted inhibitor of complement protein C5, is currently used to treat atypical hemolytic uremic syndrome. One case report describes a patient with severe preeclampsia/HELLP syndrome at 26 weeks, treated with eculizumab, who experienced clinical improvement, normalization of laboratory studies, and prolongation of pregnancy for 17 days [55]. In contrast, five pregnancies in which eculizumab was used to treat atypical hemolytic uremic syndrome did not show uniform benefit [56]. Though eculizumab and other complement inhibitors may play a role in obstetric APS, more investigation is needed.

In summary, the impact of treatment on pregnancy in women with obstetric APS has not been tested in well-designed trials. Certain clinical characteristics are associated with poor pregnancy outcomes despite treatment with antithrombotic agents. Understandable concerns about the risk of thrombosis during pregnancy, particularly in women with a history of thrombosis and or SLE, make trial design difficult. High rates of adverse pregnancy outcomes despite antithrombotic therapy in women who are repeatedly positive for LA or triple positive call for well-designed trials of novel therapies.

Group Conclusion

Treatment of patients with obstetric APS remains controversial. It is still based on LDA and heparin, although randomized trials have not supported these conclusions. Different populations, with mixed manifestations of the syndrome and unequal laboratory criteria, small number of patients, and difficulty of randomizing patients with obstetric morbidity are the causes of controversy. Studies for new promising therapies should strictly follow international consensus for APS [12], ideally in a multicenter effort.

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Chapter 13
Treatment of Non-criteria Manifestations in Antiphospholipid Syndrome

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Introduction

Non-criteria manifestations of antiphospholipid syndrome (APS), such as skin ulcers, antiphospholipid antibody (aPL)-associated nephropathy, thrombocytopenia, and heart valve disease, represent a treatment challenge. Only few formal prospective studies address their pathogeneses and treatments. The management of some manifestations of APS remains empirical and with limited evidence-based data.

In this chapter, together with a concise literature review, each situation is illustrated by a real clinical case followed by an expert discussion. Potential future treatment strategies for aPL-positive patients are also discussed in this chapter.

Skin Ulcers and Livedoid Vasculopathy

Literature Review

Livedoid vasculopathy (LV) is a rare dermatological condition characterized by recurrent painful ulcerations that generally heal as porcelain-white, atrophic, stellate scars (*atrophie blanche*). The ulcers may recur cyclically in a seasonal fashion. Livedoid vasculopathy is classified as a "reticulate eruption," given the netlike pattern of pigmentation seen on the lower limbs. First described in 1967 by Bard and Winkelmann, the condition is known by a host of names, including livedo reticularis

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with summer or winter ulcerations, painful purpuric ulcers with reticular pattern of the lower extremities (PURPLE), segmental hyalinizing vasculitis, or livedoid vasculitis [1, 2].

Given the lack of a true histological vasculitis, LV is more accurately categorized as cutaneous vasculopathy rather than as vasculitis. Hence the preferred name for this condition is livedoid vasculopathy [1, 2].

Although the disease seems to behave as procoagulant disorder, the etiology of LV is unknown. Investigations and treatment strategies have largely focused on identifying and managing an underlying hypercoagulable state. Various abnormalities of coagulation are associated with LV, including factor V Leiden mutation, protein C deficiency, increased plasma homocysteine, abnormalities in fibrinolysis, and increased platelet activation. Autoimmune disorders such as APS, systemic lupus erythematosus (SLE), and cold-precipitated proteins (cryoglobulin, cryofibrinogen, and cold agglutinin) should also be considered [1–3]. Necrotic skin ulcerations have been reported since 1963 in association with lupus anticoagulant (LA) [4]; the prevalence of LV in APS patients is approximately 5% [5].

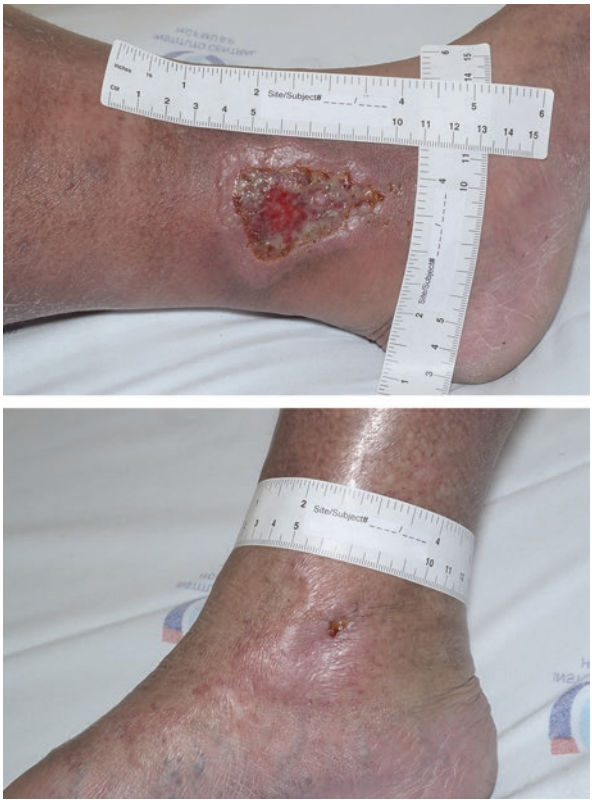
The key investigation to confirm the clinical diagnosis and to exclude other causes is a skin biopsy. Multiple samples should be obtained from affected intact purple skin (not ulcers). Typical histological features include segmental hyalinization, endothelial cell proliferation, and intravascular fibrin deposition, but not neutrophilic vasculitis [1, 2]. Thrombi within lumen and blood extravasation may be present; the absence of a perivascular infiltrate or leukocytoclasia argues against a true vasculitis [6, 7].

Diffuse cutaneous necrosis due to microvasculature thrombosis can be a therapeutic dilemma, and many different treatment approaches have been used [8]. In isolated LV lesions, combination therapy with low-dose aspirin and dipyridamole or pentoxifylline has been effective in some cases [9, 10]. If lesions recur or extend despite antiplatelet agents, oral anticoagulation is usually prescribed [11–13]. Anti-vitamin K is the best option since it is effective in preventing new thrombotic events in APS patients [13]. A phase II multicenter trial with rivaroxaban for LV concluded that rivaroxaban with enoxaparin is an option [14]. Other possible treatments include sildenafil, tissue plasminogen activator (rTPA), intravenous immunoglobulin (IVIG), and plasma exchange. No randomized double-blind control trials support their use, but these drugs have been effective in anecdotal cases [15–18]; high-dose IVIG improved pain and ulcerations in LV resulting in improvement in quality of life in 10/11 cases in one series [19]. Lesions are usually refractory to corticosteroids and immunosuppressive therapy [6].

Some case reports describe successful treatment with rituximab [20, 21]. Erkan et al. in a 12-month, phase II pilot study of rituximab therapy (RITAPS) in adults concluded that rituximab may control some, e.g., refractory skin ulcers, non-criteria manifestations [22].

Case Presentation

A 40-year-old woman with primary APS (two pregnancy losses, three deep venous thromboses [DVT], one pulmonary thromboembolism, and persistently positive LA test) presented with painful small stellated skin ulcers on the medial malleolus of left leg (Figs. 13.1 and 13.2). She had been taking anti-vitamin K anticoagulants for 3 years and had used compression stockings since the first DVT, but she had stopped using the stockings after the first skin ulcer due to pain. Her international normalized ratio (INR) was frequently out of target. She also had diabetes, obesity, hypertension, and hypothyroidism. LV, post-thrombotic syndrome, hypertensive ulcer, and diabetic ulcer were also considered in the differential diagnosis. Vascular surgery assessing the symptoms (using Villalta score) and performing a duplex ultrasound, suggested treating the ulcers as LV. Despite close follow-up, low-dose aspirin, dipyridamole, diosmin (prolongs the vasoconstrictor effect of norepinephrine), and pentoxiphylline in addition to anticoagulation, she developed new ulcers. She was intolerant to hydroxychloroquine. The treatment was changed from warfarin to enoxaparin, but the patient's lesions remained refractory.



Figs. 13.1 and 13.2 Antiphospholipid antibody-associated skin ulcers

Expert Opinion 1 (Paulo Ricardo Criado, Dermatology)

Livedoid vasculopathy is a recurrent painful ulcerative disorder, on the skin of the lower extremities, associated with coagulation disorders, autoimmune connective tissue diseases, paraproteinemia, and neoplasia [23, 24]. It results from dermal capillary and small-vessel thrombosis and/or insufficient fibrinolysis due to endothelial, platelet, or coagulation dysfunction [3].

Dermatological manifestations of APS are classified as thrombotic or non-thrombotic. Thrombotic manifestations present as necrotic ulcers from different diseases like LV, Degos disease, pyoderma gangrenosum-like ulcers, necrotizing purpura, thrombophlebitis, periungual ulcerations, multiple linear subungual hemorrhages, digital gangrene and disseminated superficial cutaneous necrosis, and purpura. Non-thrombotic manifestations include livedo reticularis, livedo racemosa, acrocyanosis, primary anetoderma, blue finger syndrome, chronic pigmentous purpura, and chronic urticaria [25, 26].

The diagnosis of LV is made by a deep skin biopsy that includes the epidermis, dermis, and hypodermis, since cutaneous polyarteritis nodosa (cPAN) must be excluded. (In cPAN subcutaneous leukocytoclastic arteritis may be missed in a superficial skin biopsy.)

The treatment of LV is heterogeneous. There are no prospective and controlled studies, and all agents or interventions used are off-label. Our dermatology study group in Brazil proposes an individualized sequential step-by-step treatment (Table 13.1) and is based on the cost-effectiveness and risk-benefit options.

In conclusion, LV is a dermatological manifestation of APS; a correct clinical and histopathological diagnosis is necessary to optimize treatment. Many off-label drugs are used to treat this condition. Controlled studies will be necessary [32].

Table 13.1 Proposed step-by-step management algorithm for livedoid vasculopathy

Step 1	Stop smoking or nicotine patches Treat the underlining thrombophilia (if APS: acetylsalicylic acid / anticoagulation) Treat venous stasis [diosmin + hesperidin (flavonoids mixture with phlebotonic properties) + pentoxifylline and stockings if not contraindicated + vascular surgery referral] [27] Use analgesic drugs (such as tramadol, gabapentin, or pregabalin) [28]
Step 2	Add acetylsalicylic acid (if not already used) [9, 10], dipyridamole, cilostazol, and/or hydroxychloroquine
Step 3	Add hyperbaric oxygen therapy [29] and/or rivaroxaban [14]
Step 4	Add danazol [30] or immunosuppressive drugs (as rituximab) [20] or intravenous immunoglobulin [31] or tissue plasminogen activator (tPA) [17]

Expert Opinion 2 (Kurosh Parsi, Dermatology)

The patient presented here seems to have been appropriately diagnosed with LV; however, the diagnosis needs to be confirmed by a skin biopsy. Vascular studies, including venous incompetence studies, should also be performed to exclude coexistent venous hypertension. Differential diagnosis includes venous ulcers, arterial ulcers, lupus panniculitis, pyoderma gangrenosum, skin neoplasms such as squamous cell carcinoma, embolic events, and deep fungal or mycobacterial infections [1, 2].

Treatment of LV is challenging and may require treatment of both the associated coagulopathy and venous hypertension. General measures include avoiding temperature variations that trigger the ulceration. The associated venous hypertension gets worse in heat while vasospasm of microvessels gets worse in cold. Hence both extremes of temperatures result in worsening of the condition [1, 2].

Chronic venous insufficiency results in stagnation of blood flow in the superficial venous network and a predisposition to thrombosis. Improved outcomes have been reported with compression therapy. The best grade of compression is class II (20–30 mmHg), unless contraindicated due to associated peripheral arterial disease. Ankle brachial index measurements and arterial duplex studies may be required in high-risk patients. Treatment for venous disease, using a combination of intravenous laser ablation and foam sclerotherapy, may heal a patient's ulcers and clear skin pigmentation. Treatment of associated venous hypertension may expedite the healing of ulcers and almost complete clearance of the associated pigmentation (manuscript in press).

Many of the documented treatments for LV focus on anticoagulant therapy with warfarin, heparin, or low-molecular-weight heparin (LMWH) and rivaroxaban [13, 14]. The evidence is mostly anecdotal and based on small case series. Antiplatelet agents, all used off-label, and which include aspirin, clopidogrel, ticlopidine, pentoxifylline, and dipyridamole, have been tried with varying success [1, 2, 10].

The patient in the case has been refractory to anticoagulation, which does not heal ulcers. She should use class II compression and she should be assessed for venous disease, which, if present, should be treated with laser ablation and foam sclerotherapy. The latter is useful to ablate abnormal vessels in the region of ulceration.

Oral anti-inflammatory drugs, such as oral corticosteroids and non-steroidal anti-inflammatory drugs, and antineutrophilic agents such as colchicine, have a role in the management of patients with an underlying inflammatory disease. Other proposed treatments, based on anecdotal experience, include nicotinic acid, hyperbaric oxygen, calcium channel blockers, L-arginine, IVIG, and danazol. Severe cases have responded to tissue plasminogen activator and to intravenous immunoglobulin [1, 2, 17, 18], all off-label uses.

Livedoid vasculopathy is relatively easy to diagnose but challenging to manage. Treatment should reduce the thrombotic load in the dermal microvasculature. Affected patients should be referred to a specialist, such as a vascular physician or a vascular dermatologist. Venous hypertension should be actively treated.

Severe Thrombocytopenia

Literature Review

Thrombocytopenia is the most common hematological manifestation of APS, with a frequency ranging from 20% to 50%. The differences in prevalence reported in studies depend mostly on different threshold descriptions of thrombocytopenia. In most cases, thrombocytopenia is mild (50–150 × 10^9/L); severe thrombocytopenia (<20 × 10^9/L) is rare. Even in the latter cases, hemorrhage is far less common than thrombosis [33, 34].

In a retrospective study of 44 thrombocytopenic patients with aPL, bleeding did not occur, and 14 (32%) had thrombotic events. In an Italian registry of aPL, although 25% of 319 patients with APS had thrombocytopenia, only four suffered severe bleeding [34]. Similarly, in 32 patients with severe thrombocytopenia (<50 × 10^9/L), three had thromboses and two had hemorrhage [35].

The pathogenesis of thrombocytopenia in APS is potentially multifactorial [36]. In animal models and in vitro studies, aPL binds and activates platelets; thus, an aPL-mediated platelet destruction may contribute to thrombocytopenia in APS patients. On the other hand, severe thrombocytopenia correlates more closely with antiplatelet glycoprotein (GP) antibodies than it does with aPL. Antibodies directed against platelet surface GPs have been identified in 40–70% of thrombocytopenic patients with APS, similar to what is seen in idiopathic thrombocytopenic purpura (ITP) [37, 38].

Because there are no guidelines for treatment of APS-associated thrombocytopenia, the ITP guideline is used as a reference [39]. Treatment is necessary in cases of severe thrombocytopenia (<20 × 10^9/L) or of bleeding. Glucocorticoids, IVIG, immunosuppressive therapies (azathioprine, cyclophosphamide), danazol, and hydroxychloroquine are possible and effective therapies [36, 40–43]. Rituximab may be an alternative in refractory thrombocytopenia. In a literature review, 30% cases treated with rituximab had complete response and 40% a partial improvement of platelet counts [43]. Splenectomy is an option for refractory cases; however, because of surgery-associated thromboses, this should be considered with extreme caution in APS patients [44, 45].

Although some case reports state that eculizumab, a terminal complement inhibitor, is effective for treating catastrophic APS (CAPS) and thrombotic microangiopathy in APS patients [46, 47], there are no data to support its use in other forms of APS-related thrombocytopenia.

Eltrombopag and romiplostim are thrombopoietin receptor agonists, approved for management of chronic ITP. Despite their efficiency in rapidly raising platelet levels, recent case reports have showed severe thrombotic events (including CAPS, and deaths) after its administration in patients with aPL [48–50]. We believe that treatment with thrombopoietin receptor agonists should not be used in patients with APS patients.

Before introducing anticoagulants, platelet levels should be higher than $>50 \times 10^9$/L [35]. A difficult dilemma is to diagnosis a new thrombotic event in an APS patient with severe thrombocytopenia ($<30 \times 10^9$/L). Failure to address the clot may be life-threatening, while anticoagulation may lead to hemorrhagic complications. Low-dose anticoagulation with unfractionated heparin associated with immunosuppressant may be an option [51].

Case Presentation

A 34-year-old woman, previously healthy, was admitted because of severe chest pain. The electrocardiogram revealed ST elevation, and she was promptly taken to coronary catheterization. The coronary study showed thrombi in the circumflex coronary and excluded atherosclerotic coronary disease. The thrombus was aspirated; no stent was placed. She received anticoagulation and antiplatelet drugs and was sent to the intensive care unit. Surprisingly, the first laboratory results from the emergency room showed a platelet count of 7×10^9/L. Further investigation revealed a strongly positive LA and high titer anticardiolipin antibody (aCL) IgG. Anti-β_2-glycoprotein-I antibody (aβ_2GPI) was negative. The rheumatology team decided to discontinue antiplatelet drugs and keep the unfractionated heparin, with a strict control. She also received methylprednisolone (1 mg/kg) and IVIG with an improvement in platelet levels to 85×10^9/L. The patient was discharged with stable platelet levels (around 80×10^9/L) on warfarin, prednisone, and hydroxychloroquine. During hospitalization, virus infection, thrombotic thrombocytopenic purpura, bone marrow disorders, systemic lupus erythematosus, and other collagen diseases were excluded. After 3 months, repeat aPL tests confirmed the double positivity and the APS diagnosis. This patient is now taking only warfarin and hydroxychloroquine, has a higher platelet count, and has had no recurrence of thrombosis.

Expert Opinion (Reyhan Diz-Kucukkaya, Hematology)

Platelets are the major cellular component of a thrombus, especially in arterial thrombosis; development of thrombosis in a patient with severe thrombocytopenia is rare. Antiphospholipid syndrome, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, heparin-induced thrombocytopenia-thrombosis, and acute leukemia are possible causes [52]. Interestingly, patients with ITP have an increased risk of thrombosis, but, even in severe thrombocytopenia, hemorrhage is far less common than is thrombosis, as in the present case. Several explanations have been postulated for the development of thrombosis in patients with ITP including the presence of aPL itself, activation of the complement system, and individual risk factors for thrombosis [36, 53, 54].

Immunosuppressive therapies may decrease titers of antiplatelet antibodies and increase platelet counts in APS patients with thrombocytopenia, but they do not reduce the titers of aPL [55], suggesting that thrombocytopenia is a secondary phenomenon in at least some APS patients. Thrombotic microangiopathy may contribute to both thrombocytopenia and thrombosis in a subset [56].

Severe thrombocytopenia in patients with APS is treated similarly to that in patients with ITP; it usually responds well to glucocorticoids and immunosuppressive drugs. Although IVIG may increase the platelet count very rapidly in these patients, the thrombotic risk of IVIG itself should be considered [57]. The data concerning the use of thrombopoietin receptor agonists in patients with APS is limited to case reports and was associated with an increased risk of thrombosis [50, 58].

The choice and monitoring of anticoagulant therapy in APS patients with severe thrombocytopenia and thrombosis are also challenging. Although unfractionated heparin is preferred in patients with acute coronary syndrome, activated thromboplastin time and activated clotting time are prolonged in patients having LA; thus, anti-factor Xa and protamine titration assays are recommended for heparin monitoring. The use of low-molecular-weight heparin is appealing in treating patients with LA, since it causes a more predictable anticoagulant effect [59].

Although most patients with aPL and thrombocytopenia will not require treatment, some, as in this case, represent a treatment challenge. Corticosteroids and immunosuppressants should be considered when clinically significant thrombocytopenia occurs.

Cardiac Valve Disease

Literature Review

Cardiac valve disease (CVD), a non-criteria manifestation of APS, is defined as (a) valve thickness >3 mm, (b) localized thickening involving the proximal or middle portion of the leaflets, or (c) irregular nodules on the atrial face of the mitral valve and/or the vascular face of the aortic valve [60]. Although most of the cases are asymptomatic [61], about 5% of patients with valve disease will progress to cardiac failure, requiring valve replacement [60]. Additionally, a meta-analysis showed that 48% of aPL-positive lupus patients have valve disease compared with only 21% of aPL-negative SLE patients [62].

Valve lesions are associated with high risk of arterial events in primary APS patients [63]. A meta-analysis of 23 studies, including 1656 patients with SLE and 508 cases of valve disease, showed a threefold higher frequency for any valve lesion in SLE patients with aPL, compared to those without aPL. The risk associated with IgG aCL was as high as for LA [64].

On histopathology, these lesions are characterized by superficial and intravalvular deposits of fibrin with subsequent organization [63]. A mechanism implicated in the generation of valve lesions is that an immune-mediated endothelial activation by aPL triggers an inflammatory cascade, resulting in complement deposition and valve damage [60].

According to the 13th International Congress on Antiphospholipid Antibodies Task Force on CAPS and Non-criteria APS Manifestations, transthoracic echocardiogram (TTE) in APS patients with thrombosis (mainly arterial) is recommended. In patients with normal valves and in the absence of atherosclerotic risk factors, follow-up may be unnecessary. If valve lesions exist, serial echocardiographic follow-up is suggested [65]. According to the American College of Cardiology, transesophageal echocardiogram (TEE) should follow a non-diagnostic (transthoracic echocardiogram) TTE. However, TEE may be the initial test in patients with a suspected cardioembolic event and no history of atrial fibrillation [66]. Two-dimensional echocardiography studies are the standard modality for diagnosis [67, 68]. Transesophageal echocardiogram is more accurate for detection of vegetations and thickening compared to Transesophageal echocardiogram (73% vs. 39%) [69].

A study comparing different techniques for cardiac assessment in APS concluded that cardiovascular magnetic resonance (CMR) identifies a high prevalence of occult myocardial scarring and endomyocardial fibrosis in APS. If quantification of heart valve disease and stress myocardial perfusion-fibrosis is needed, CMR is the technique of choice [70].

The optimal treatment of aPL-related cardiac valve disease is unknown. Oral anticoagulant treatment with an INR goal between 2.0 and 3.0 and aspirin (100 mg/day) was not effective for valve lesion regression [71]. Corticosteroid treatment was not effective for improving valve healing [72]. Valve replacement in patients with APS carries significant early and late morbidity and mortality [73]. Surgical risk is even greater when active SLE and renal involvement are present [74]. The successful use of transcatheter aortic valve replacement (TAVR) was reported in a single case study of the treatment of severe aortic stenosis in a patient with active SLE and APS [75].

Case Presentations

Case 1 A 29-year-old female, taking an oral contraceptive, with a history of migraine and premature delivery at 34-week gestational age, presented with left-sided facial numbness due to a right thalamic stroke while. She was triple positive for aPL. She was started on LMWH and bridged to warfarin. Two weeks later, brain magnetic resonance imaging (MRI) revealed multiple scattered, bilateral supratentorial and infratentorial acute infarctions in the setting of a subtherapeutic INR; therefore, LMWH was reinitiated. Two days later, she reported an episode of aphasia for several hours. A new brain MRI showed multiple new foci of diffusion restriction consistent with acute infarction. Transthoracic echocardiogram was nor-

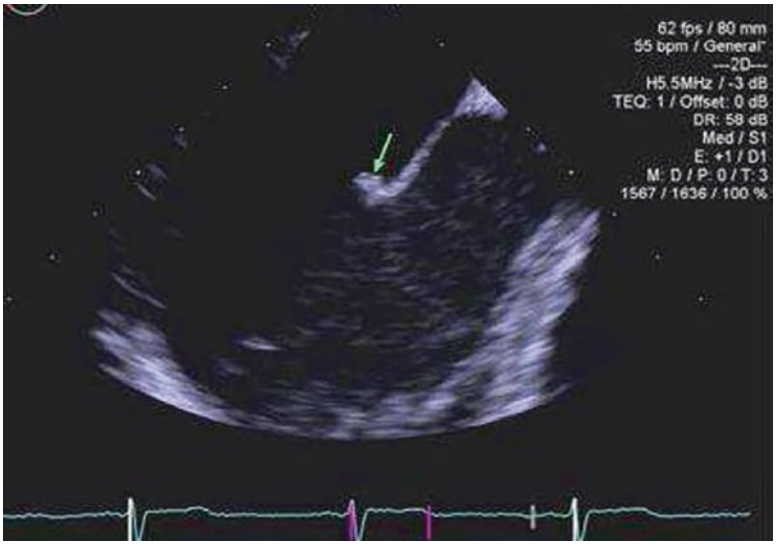


Fig. 13.3 Antiphospholipid antibody-associated cardiac valve disease; a small, sessile echodensity on the left atrial surface of the anterior mitral leaflet (Permission to publish was obtained from Arthritis Care & Rheumatology)

mal but TEE showed a sessile echo dense lesion (0.4 × 0.2 cm) on the left atrial surface of the anterior mitral leaflet (Fig. 13.3).

Case 2 A 38-year-old female, taking an oral contraceptive, with no prior history of thrombosis, presented with cyanosis of the left second finger. Ultrasonography and magnatic resonance angiography were normal; she was found to have triple aPL positivity and was started on LMWH with quick improvement of the pain and color of fingers. Transthoracic echocardiogram was normal, but TEE showed a small mobile echo-dense lesion on the aortic valve. Both patients had persistent aPL titers when repeated in 12 weeks.

Expert Opinion (Mary Carmen Amigo, Rheumatology)

Cardiovascular disease in APS patients is associated with stroke, transient ischemic attack (TIA), epilepsy, and migraine [76]. Heart failure, infective endocarditis, valve replacement, and death are all complications of valve damage irrespective of its etiology. Lupus anticoagulant positivity with mitral thickening/regurgitation is associated with a tenfold greater risk of cerebral infarcts in patients with lupus [77]. In APS, CVD is associated with an 8.4-fold risk of arterial thrombosis, as reported in a 12-year follow-up study [78].

Case 1 has APS with arterial thrombosis and a high risk of re-thrombosis. RATIO study showed that the odds ratio for ischemic stroke in women with positive LA was 43.1 (12.2–152.0) but increased to 201.0 (22.1–1828.0) if the women was taking an oral contraceptive [79, 80]. In the second case, as there is no confirmation of thrombosis or pregnancy morbidity, a diagnosis of APS based on classification criteria [81] is not possible. However, digital ischemia and an aortic valve vegetation with triple aPL positivity strongly suggest that she is a high-risk patient.

In patients with a high-risk profile, aspirin is not effective for primary thromboprophylaxis and is significantly less effective than vitamin K antagonists for secondary prevention [82, 83]. There is no consensus regarding optimal antithrombotic management of patients with ischemic stroke/TIA and aPL (independent of valve disease). We look forward to data from ongoing studies on the efficacy and safety of the new oral anticoagulants in patients with APS.

In case 1, a long-term oral anticoagulation (INR > 3.0) or long-term oral anticoagulation (INR 2.0–3.0) with low-dose aspirin (100 mg/day) is the best approach [84–86]. Some investigators suggest that D-dimer level is an indicator of a higher susceptibility to embolism recurrence [87]. Literature on atrial fibrillation patients demonstrates that patients with elevated D-dimer during oral anticoagulation therapy are at high risk for thromboembolic events [88].

In case 2, as digital ischemia is a potentially serious complication, it requires a prompt assessment and introduction of treatment. The initiation of LMWH promptly improved pain and color of the fingers. Treatment alternatives include LMWH, warfarin (INR > 3), or warfarin (INR 2.0–3.0) plus low-dose aspirin [84].

Even though there is no evidence-based data, HCQ or statins can be considered as additional treatments [43]. In a small cohort of refractory cases, IVIG proved to be beneficial [89]. Cognitive dysfunction, a common and serious complication in patients with cerebral ischemia, merits consideration of rituximab, which may benefit non-criteria manifestations of APS such as thrombocytopenia, skin, and valve disease [22]. Scant data regarding corticosteroids for aPL-associated valve disease suggest they are not effective [90, 91].

Prospective studies show that antithrombotic and/or antiplatelet treatment does not stop valve disease progression [71, 92, 93]. However, antithrombotic treatment should be given to prevent emboli, according to current guidelines [94]. There is no consensus on the treatment of valve disease itself.

Antiphospholipid Antibody-Associated Nephropathy

Literature Review

Antiphospholipid antibody-associated nephropathy is a non-criteria APS manifestation [81]. This small-vessel nephropathy, called APS- or aPL-associated nephropathy, was first described in primary APS [95] and further described in SLE patients with positive aPL, with and without APS [96].

Antiphospholipid antibody-associated nephropathy is characterized by thrombotic microangiopathy (the acute lesion) and chronic vaso-occlusive lesions, such as fibrous intimal hyperplasia, organizing thrombi and/or fibrous occlusions of arteries or arterioles and focal cortical atrophy. Other causes of renal microangiopathy such as malignant hypertension, diabetes mellitus, thrombotic thrombocytopenic purpura, and systemic sclerosis [65, 81] should be excluded.

The major clinical characteristics of this nephropathy include mild-to-severe hypertension, microscopic hematuria, proteinuria (mild to nephrotic level), and renal insufficiency. The latter is usually mild but may progress to renal failure [97].

The 13th International Congress on Antiphospholipid Antibodies Task Force on Non-criteria APS Manifestations critically evaluated studies on the relationship between aPL and aPL-associated nephropathy [65] and concluded that, among primary APS patients, aPL-associated nephropathy accounted for 90–100% of all biopsy-proven renal involvement and for 67–100% of patients with SLE-APS who had renal involvement [65, 96].

There is no consensus on the management of aPL-associated nephropathy patients, which may occur despite full-dose anticoagulation and may not improve if anticoagulation/antiplatelet therapy is initiated after diagnosis. Empirical options are primarily focused on the management of hypertension and proteinuria [98]. Based on case reports or small case series, angiotensin-converting enzyme (ACE) inhibitors, aspirin, oral anticoagulants, hydroxychloroquine, corticosteroids, and/or immunosuppressive agents can be alternatives [99–101].

In patients with aPL but without APS, aspirin and/or hydroxychloroquine can be considered, especially in patients with SLE. Oral anticoagulants may be used in patients with high-risk aPL profile, such as those with persistently positive LA, persistently positive aCL in medium-high titers, and those with triple positivity [102]. The control of blood pressure and proteinuria, using mainly ACE inhibitors, is recommended for all the patients with aPL-associated nephropathy [96]. Other treatment options for more refractory cases, based on few studies, include IVIG [89], rituximab [22], and eculizumab [85, 103].

Case Presentation

A 21-year-old woman with APS (triple positive aPL, prior dural venous sinus thrombosis requiring ventriculo-peritoneal shunt, DVT extending to the right common and deep femoral veins, chronic occlusion of the celiac and right external iliac artery, and ulcer on the left tibia) developed moderately elevated blood pressure. Laboratory tests confirmed persistent proteinuria of 1.8 g/24 h and active urine sediment with normal serum creatinine levels. The patient's left lower extremity ulcer worsened with painful superficial ulcerations of the skin bilaterally and development of necrosis requiring surgical debridement. A repeat skin biopsy was consistent with small-vessel vasculopathy. Renal biopsy demonstrated aPL-associated

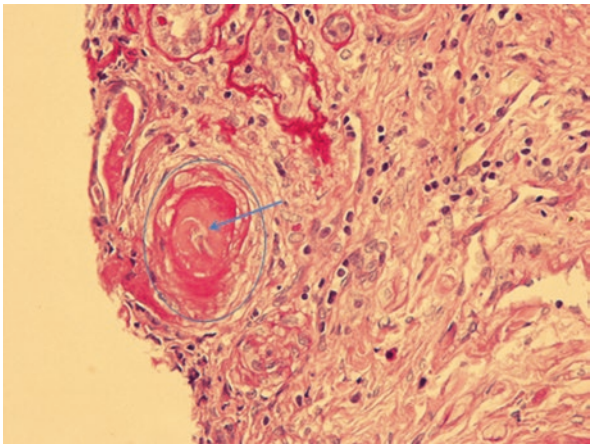


Fig. 13.4 Antiphospholipid antibody-associated nephropathy; renal arteriole occluded by a thrombus (*arrow*). Pas stain ×200

nephropathy lesions (Fig. 13.4). Treatment with rituximab was initiated, with slowly decreasing 24-h urinary protein levels, stable renal function, and gradual improvement and ultimate healing of the leg ulcers.

Expert Opinion (Maria Tektonidou, Rheumatology)

Currently, there is no consensus on treatment of aPL-associated nephropathy. Studies of SLE patients show that vascular lesions, especially thrombotic microangiopathy in lupus nephritis, are associated with poor renal outcomes, such as doubling of serum creatinine or end-stage renal disease [104]. The role of anticoagulation was not analyzed in the studies of aPL-associated nephropathy among patients with lupus nephritis because of the limited number of patients on anticoagulation. According to the European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for lupus nephritis management, despite the lack of evidence from controlled studies, hydroxychloroquine and/or antiplatelet/anticoagulant treatment can be considered in combination with immunosuppressive treatment [105]. Appropriate management of hypertension and effective control of proteinuria with ACE inhibitors and angiotensin receptor blockers are strongly recommended.

Patients with renal involvement and aPL-associated nephropathy who fulfill the criteria for definite APS should be treated for APS. The question about best treatment remains open for patients with no history of vascular thrombosis or pregnancy morbidity. Antiphospholipid antibody-associated nephropathy is included as a non-criterion manifestation of APS; the evidence for its inclusion among the definite criteria of APS was considered as moderate by the 14th International Congress on Antiphospholipid Antibodies Task Force on APS Clinical Features [106]. The use of lifelong anticoagulation in asymptomatic aPL carriers who develop APS nephropa-

thy remains controversial. Previous case series showed successful treatment with oral anticoagulants in some patients with clinical and histological manifestations of aPL-associated nephropathy [107, 108]. Intravenous immunoglobulin and/or plasmapheresis has been used in severe or refractory cases [109]. Plasmapheresis and rituximab were used together successfully in a patient with severe APS and nephropathy [109]. In an open-label pilot trial (RITAPS study) of patients with non-criteria manifestations, rituximab was associated with partial improvement in one adult with aPL-associated nephropathy [22]. A task force report on APS syndrome treatment trends stated that B-cell inhibition may have a role in difficult-to-treat patients, possibly in those with hematologic and microthrombotic/microangiopathic manifestations [85].

Pravastatin, which downregulates tissue factor, prevented glomerular injury in mouse model of thrombotic microangiopathy that used both mouse and human aPL [110]. In vitro and in vivo studies showed the importance of complement activation in pathogenesis of APS, and clinical studies demonstrated the efficacy of complement inhibition in the "thrombotic microangiopathy" group of disorders characterized by thrombocytopenia and microangiopathic hemolytic anemia (intravascular hemolysis and presence of peripheral blood schistocytes), such as the paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, and CAPS [111]. The C5a inhibitor eculizumab has also been effective in patients with CAPS and in cases with kidney posttransplant thrombotic microangiopathy associated with APS [112, 113]. Recent data show that mechanistic target of rapamycin (mTOR) activation is involved in the vascular lesions associated with APS [114]. Patients with aPL-associated nephropathy needing transplantation who were pretreated with an mechanistic target of rapamycin (mTOR) inhibitor had no recurrence of vascular lesions and had decreased vascular proliferation on biopsy, suggesting a potential role of mTOR inhibition in the treatment of aPL-associated nephropathy. There are no studies reporting on their efficacy in aPL-associated nephropathy.

Group Conclusion

Skin ulcers, aPL-associated nephropathy, thrombocytopenia, and heart valve disease represent a treatment challenge, and their management remains empirical and with limited evidence-based data. Here we summarize the expert's opinion:

- A suitable diagnosis of livedoid vasculopathy requires a deep skin biopsy. In addition to an adequate anticoagulation, venous insufficiency should be strictly treated. Other medications such as aspirin, dipyridamole, immunoglobulin, and rituximab can be used in refractory cases.
- Severe thrombocytopenia in patients with APS is treated similarly to that in patients with ITP, and it usually responds well to glucocorticoids and immunosuppressive drugs.
- Prospective studies show that antithrombotic and/or antiplatelet treatment do not stop valve disease progression; however, antithrombotic treatment should be given to prevent emboli.

- There are several potential medications being studied for the treatment of aPL-associated nephropathy. Rituximab, eculizumab, mTOR inhibitor, and pravastatin are some of the candidates.

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Chapter 14
Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION)

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Background

Since the description of antiphospholipid syndrome (APS) [1] in the mid-1980s, APS clinical research has grown exponentially. In 2010, the 13th International Congress on Antiphospholipid Antibodies (aPL) Organizing Committee, chaired by the late Dr. Silvia Pierangeli, created a Clinical Research Task Force (CRTF), with the objectives of evaluating limitations and developing guidelines for investigators to improve APS clinical research. The CRTF recommended an international collaborative network to design and conduct prospective, large-scale, multicenter clinical trials in individuals or patients with aPL [2]. An "APS CRTF Summit" in November 2010 generated ideas for future collaborative clinical trials and thus, began an international APS clinical research network entitled "Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION)" (www.apsaction.org) [3].

The founding principle of APS ACTION is international collaboration and data sharing. A secondary objective is to refine and advance definitions of aPL-associated clinical manifestations. The initial organizational steps and accomplishments of APS ACTION have been published elsewhere [3, 4]. This paper provides a summary of APS ACTION's organizational structure, initiatives, ongoing and completed research efforts, and future directions.

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Organizational Structure and Initiatives

Membership

To date, the network is composed of 53 physician-scientists, from multiple disciplines, from 31 international centers, who are interested in APS research (Table 14.1). The APS ACTION Executive Committee is composed of eight elected members, representing different regions of the world. Honorary members comprise a small, select group of individuals who have made major contributions to the field of APS and/or have demonstrated extraordinary service to APS ACTION.

Core Laboratories

Five worldwide APS ACTION core laboratories have been created; they are located in São Paulo (Brazil), Sydney (Australia), Galveston (USA), Padova (Italy), and London (UK). The purpose of standardized core laboratories is to improve comparability of test results for use in clinical trials and research studies.

Table 14.1 APS ACTION center locations and members

Austria: Graz (Karolina Mayer-Packel)
Australia: Sydney (Bill Giannakopoulos, Steve Krilis)
Brazil: Rio de Janeiro (Guilherme de Jesus, Roger Levy), São Paulo (Renata Rosa, Danieli Andrade)
Canada: Quebec (Paul F. Fortin)
China: Beijing (Zhouli Zhang)
France: Nancy (Stephane Zuily, Denis Wahl)
Greece: Athens (Maria Tektonidou)
Italy: Brescia (Cecilia Nalli, Laura Andreoli, Angela Tincani), Milan (Cecilia B. Chighizola, Maria Gerosa, Pierluigi Meroni), Padova (Alessandro Banzato, Vittorio Pengo)
Jamaica: Kingston (Karel De Ceulaer)
Japan: Sapporo (Tatsuya Atsumi)
Lebanon: Beirut (Imad Uthman)
Netherlands: Utrecht (Ronald Derksen, Philip de Groot)
Spain: Barakaldo (Guillermo Ruiz Irastorza), Barcelona (Ignasi Rodriguez-Pinto, Guillermo Pons-Estel, Ricard Cervera), Madrid (Esther Rodriguez)
Poland: Warsaw (Ewa Haladyj)
United Kingdom: London (Ian Mackie, Hannah Cohen, Maria Efthymiou; and Savino Sciascia, Maria Laura Bertolaccini, Maria Cuadrado, Giovanni Sanna, Munther Khamashta)
USA: Baltimore (Michelle Petri), Boston (Medha Barbhaiya), Chapel Hill (Robert Roubey), Chicago (Jason S Knight), Durham (Tom Ortel), Galveston (Emilio Gonzalez, Rohan Willis), New York City (Steven Levine, Jacob Rand, H Michael Belmont, Doruk Erkan, Jane Salmon, Michael Lockshin), Salt Lake City (Ware Branch)

Annual Scientific Summits and Workshops

Annual workshops and summits since 2010 have served as a forum for collaboration and critical organizational planning by (a) finalizing the organizational structure, (b) discussing administrative and logistical issues, (c) developing and discussing the progress of research projects, (d) providing continuous data entry and specimen collection training and (e) conceptualizing spin-off clinical projects. During the 15th International Congress on aPL (www.apsistanbul2016.org), APS ACTION co-organized a session with the scientific planning committee of the congress in which international/national collaborative APS research efforts and strategies to recruit patients for rare diseases were discussed.

Young Scholar Initiative and Exchange Program

As part of an effort to attract young talent to APS research, APS ACTION established the annual APS ACTION Young Scholar Program Award that recognizes junior physician-scientists, nominated by APS ACTION members, who have contributed to APS research. The APS ACTION Young Scholar Exchange Program hopes to incentivize young physicians and/or scientists, by integrating them to our community, to perform APS-related basic or clinical research.

Ongoing and Completed Research

In early 2012, APS ACTION launched two collaborative international projects: (a) a web-based clinical database of aPL-positive patients with or without systemic autoimmune diseases, including a repository with baseline and annual sample collection for future mechanistic studies and (b) a randomized controlled trial of hydroxychloroquine in primary thrombosis prevention in persistently aPL-positive, thrombosis-free patients with or without other systemic autoimmune diseases. Another focus of APS ACTION is to conduct epidemiologic studies that investigate associations among aPL tests, risk factors, and clinical outcomes that, we hope, will generate hypotheses that lead to new basic and translational research.

APS ACTION International Clinical Database and Repository (Registry)

An international, multicenter clinical database and repository (registry) allows us to study the natural history of aPL-positive patients prospectively. Data are collected and managed using REDCap electronic data capture tools hosted at Weill Cornell

Medicine [5], a secure, web-based application that supports data capture for research studies. As of November 2016, 25 centers have received Institutional Board (IRB) approval to participate in the APS ACTION registry, and 668 patients have been enrolled. Since its creation, several preliminary analyses have been performed (including those presented during the 15th International Congress on aPL). These analyses demonstrate:

- An association of triple-positive aPL profile with catastrophic APS and with cardiac valve disease, but not with other aPL-related manifestations [6].
- One-year recurrent and first thrombosis risks among persistently aPL-positive patients is 1.7% and 0% per year, respectively [7]; during extended follow-up (720 patient-years, mean 1.7 ± 0.65 years), the risk increased to 2.4% and 1.9%, respectively. The risk is associated with lupus anticoagulant (LA) and/or triple aPL positivity in addition to other non-aPL thrombosis risk factors [8].
- Cluster analysis distinguishes among patients with aPL with these different clinical phenotypes: pregnancy morbidity, cardiovascular risk factors, aPL profile, and lupus [9].
- Risk factors for future thrombosis after an aPL-related episode of pregnancy morbidity include earlier age during the first episode, selected cardiovascular (CVD) risk factors, non-criteria aPL manifestations, and positive LA test [10].
- Higher Global APS Score (GAPSS) predicts future thrombosis risk after an aPL-related pregnancy morbidity [11].
- SLE patients more often have IgA anti-β_2-glycoprotein-I ($a\beta_2$GPI) than they do IgA anticardiolipin antibody (aCL); however, IgG, IgM, or IgA aCL/$a\beta_2$GPI do not distinguish among patients with different aPL-related clinical events or among patients with and without SLE [12].
- Persistent thrombocytopenia and autoimmune hemolytic anemia occur more often in aPL-positive patients with SLE than those without SLE [13].
- Hypertension and smoking are more common in aPL-positive patients with SLE than in those without SLE; the frequency of other CVD risk factors is similar in the two groups [13].
- Antiphospholipid antibody-positive patients with serological but no clinical features of SLE are more likely to receive hydroxychloroquine (HCQ); approximately one-third of primary aPL/APS patients receive HCQ [14].
- Out of 428 thrombotic APS patients included in the registry, 19 receive (or had received) a direct oral anticoagulant (DOAC), mostly rivaroxaban. Six patients developed recurrent events during the 2-year follow-up; however, in 11/19 of patients, the DOAC use was not as per licensed indications aside from the APS diagnosis, e.g., used for recurrent arterial thrombosis [15].

These results are preliminary and some are based on retrospective data. Future analyses will provide more definitive conclusions.

APS ACTION Core Laboratory Validation Exercises

The APS ACTION core laboratory validation exercises assess intra- and interlaboratory variability (LA test, aCL, and aβ_2GPI). The LA assay was validated based on the First International Reference Panel for LA (National Institute for Biological Standards and Control [NIBSC], UK) (negative, moderately positive, and strongly positive samples used by the core laboratories) [16, 17]. Anticardiolipin antibody and aβ_2GPI enzyme-linked immunoassays (ELISA) were validated based on blinded serum samples from low, medium, and high aPL-positive patients and from negative controls. After the completion of the validation exercises, registry samples were tested at core laboratories; there was very good categorical agreement between local laboratory aPL ELISA test results used to enroll patients and core laboratory results. This agreement increased when considering only high titer samples (>40 units) [18].

A Multicenter International Randomized Controlled Trial of Hydroxychloroquine (HCQ) in the Primary Thrombosis Prophylaxis of Persistently Antiphospholipid Antibody Positive but Thrombosis-Free Patients without Systemic Autoimmune Diseases ("HCQ Trial")

The first major clinical trial designed by APS ACTION is the international, multicenter, randomized controlled clinical trial assessing efficacy of HCQ for primary thrombosis prevention in persistently aPL-positive but thrombosis-free patients with no other systemic autoimmune diseases over a 5-year study period. The secondary objectives were to determine the thrombosis incidence rate, the effect of HCQ on mortality rate, and the effect of HCQ on aPL profile. The study, partially supported by the New York Community Trust, was closed on September 30, 2015, earlier than planned, because of a low recruitment rate exacerbated by a prolonged manufacturing shortage and a price increase of HCQ [19].

We recruited 20 patients from four of eight IRB-approved centers, of whom nine were randomized to HCQ. During a mean follow-up of 1.7 years, no patient developed thrombosis and no serious adverse event occurred. Given the small number and relatively short follow-up, the study is inconclusive. Our experience supports the fact that conducting an international RCT without pharmaceutical support is challenging. We believe prospective observational follow-up of thrombosis-free aPL-positive patients in the APS ACTION registry will provide preliminary data to help determine the efficacy of HCQ for primary thrombosis prevention [19].

The Frequency of Antiphospholipid Antibodies in General Populations with Thrombosis and Pregnancy Morbidity

An APS ACTION Young Scholar project reviewed 120 full-text papers to calculate the median frequency for positive aPL tests (LA test, aCL, and aβ_2GPI) for each outcome [20]. The aPL frequency was 6% for pregnancy loss, 14% for stroke, 11% for myocardial infarction (MI), and 10% for deep venous thrombosis (DVT). Important literature limitations included varying aCL and aβ_2GPI titer cutoffs; heterogeneous aPL profiles, e.g., all three criteria tests were included in only 11% of the papers; lack of confirmation of persistence of aPL; and retrospective study design, which may underestimate aPL prevalence, given that not all subjects with these clinical outcomes are routinely tested for these antibodies. While these efforts were a first approach to derive "real" numbers from existing literature, the frequency estimates should be taken with caution. They will need to be confirmed with appropriately designed, prospective population studies.

APS ACTION Young Scholars also conducted a systematic review assessing the association between aPL in the general population and aPL-related outcomes, i.e., pregnancy morbidity, stroke, MI, and DVT [21]. When each outcome was analyzed separately, there was an association for aPL with overall pregnancy morbidity, pregnancy loss, late pregnancy loss, severe preeclampsia, stroke, MI, and DVT. The LA test had the highest association with both obstetric and thrombotic events, a consistent finding with previous systematic reviews and meta-analyses [22]. No association emerged for aPL with early pregnancy loss, intrauterine growth restriction, preeclampsia, eclampsia, and HELLP syndrome [21].

APS ACTION investigators also investigated the association of aPL and cerebrovascular events in people younger than 50 years old. In a systematic review, based on data from 5217 patients and controls from 43 studies, APS ACTION investigators demonstrated that the overall aPL frequency for any cerebrovascular event was 17.4% (range 5–56%) [24]. Antiphospholipid antibodies increased the cerebrovascular event risk 5.48-fold (95% CI 4.42–6.79) in this population. Although this systematic review was limited by variability in test reproducibility and cutoff definitions, the findings support the conclusion that aPL is associated with stroke in young populations [23].

Future Directions

Mechanistic Studies

To increase collaboration with individuals and other organizations, APS ACTION now accepts external applications for mechanistic studies.

One of the first internal mechanistic studies, conducted by APS ACTION (principal investigator, Dr. Hannah Cohen), funded by LUPUS UK, and using the APS ACTION

repository, aims to determine prevalence of activated protein C resistance (determined by thrombin generation) in aPL-positive patients, as well as its associations with anti-protein C antibodies and severity of clinical phenotype. This study will confirm and extend previous observations that high-avidity anti-protein C antibodies and acquired activated protein C may serve as markers for a severe thrombotic APS phenotype [24–26].

Outcome Studies

APS ACTION investigators will continue to analyze registry data to answer important and clinically relevant questions, e.g., treatment outcomes in APS patients presenting with arterial thrombosis.

Interventional Studies

Dr. Tom Ortel (Duke University Medical Center, Durham, NC, USA) is the principal investigator for the National Heart Lung and Blood Institute (NHLBI)-funded U34 clinical trial planning grant for the Warfarin Withdrawal in Antiphospholipid Syndrome Study (WAR-APS). This study is a double- blind, placebo-controlled, randomized clinical trial to determine whether indefinite anticoagulation is appropriate for all patients with APS. APS ACTION expertise and patient registry will play critical roles.

Dr. Vittorio Pengo (University of Padova, Padova, Italy) is the principal investigator of the ongoing multicenter (including selected APS ACTION centers), interventional, open-label, randomized, controlled trial in which warfarin-receiving triple aPL-positive patients are randomized to continue warfarin or switch to rivaroxaban (Clinicaltrials.gov Identifier: NCT02157272).

Additional internal and external collaborative clinical trial proposals are currently being considered by APS ACTION members and are expected to be underway in the near future.

Conclusion

Over the past 5 years, APS ACTION, the first international collaboration among APS clinicians and investigators focused on conducting multicenter, randomized controlled clinical trials, has fulfilled an important need in APS research. We have grown substantially in terms of increased worldwide membership and expanded

group leadership and accumulated a large, international database and repository of aPL-positive patients. In addition, we have made progress toward facilitating international research, collaboration, data sharing, and development of core laboratories. Members continue to identify gaps and limitations in the aPL/APS literature, which APS ACTION strives to improve with prospective, large-scale studies that value early diagnosis, risk stratification, basic science research to elucidate mechanisms, and improved therapies. The goal is cure—hopefully facilitated by APS ACTION.

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Part V
15th International Congress on Antiphospholipid Antibodies Task Force Reports

Chapter 15
15th International Congress on Antiphospholipid Antibodies Task Force on Antiphospholipid Syndrome Classification Report

Stéphane Zuily, Medha Barbhaiya, Karen H. Costenbader, and Doruk Erkan

Introduction

Antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity in patients with persistent antiphospholipid antibodies (aPL). Classification of antiphospholipid syndrome (APS) for clinical trials and studies currently is based on clinical and laboratory criteria identified in the "Sapporo Classification Criteria" published in 1999 [1], validated in 2000 [2], and revised in 2006 [3], known as the Revised Sapporo APS Classification Criteria (or Sydney Criteria) [4, 5].

Given the substantial morbidity and mortality related to APS and significant limitations of the current criteria, the goal of the 15th International Congress on aPL Task Force on APS Classification is to develop new evidence-based criteria to improve APS clinical research. We employed an international, multicenter approach to capture both the wide spectrum of disease manifestations and the variability in aPL laboratory testing. We hope that these new criteria will identify patients with high likelihood of having APS and will better standardize patients for APS clinical trials and epidemiologic studies. This chapter reviews the rationale for and methodology of our new APS classification criteria development effort.

Rationale for Developing New Antiphospholipid Syndrome Classification Criteria

Antiphospholipid syndrome has a large impact on mortality [6] and morbidity, especially in terms of organ damage [7] and impaired quality of life [8]; APS has a significant public health impact. In general population of patients without apparent

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autoimmune diseases, aPL is present in 9% of patients with pregnancy losses, 14% of those with stroke, 11% of those with myocardial infarction (MI), and 10% of those with deep vein thrombosis (DVT) [9]. In the United States, aPL is associated with approximately 50,000 pregnancy losses, 110,000 strokes, 100,000 MIs, and 30,000 DVTs annually [9–15]. These estimates may be inaccurate, given the limitations of the current literature and APS Classification Criteria.

The Revised Sapporo APS Classification Criteria is suboptimal due to the lack of:

- Representation of many heterogeneous manifestations of aPL [16], such as livedo reticularis, thrombocytopenia, or heart valve disease. In addition, new insights have been gained about the association of aPL manifestations not currently part of the accepted criteria with their clinical/prognostic significance in aPL-positive patients [17].
- Stratification based on systemic lupus erythematosus (SLE) diagnosis; for instance, while aPL tests may be a useful diagnostic marker in patients with lupus, they may be less important in patients without lupus.
- Incorporation of knowledge concerning other risk factors for thrombosis; for instance, at least 50% of aPL-positive patients with thrombosis have other non-aPL risk factors [18].
- Definition of pregnancy morbidity criteria [5]; new insights associate aPL with forms of pregnancy morbidity that are not part of the criteria, for instance, fetal growth restriction [19].
- Clear definition of "positive" aPL test, persistence, and the duration of positivity.
- Representation of the risk profile associated with different aPL tests. For instance, IgM anticardiolipin antibodies (aCL) and anti-β_2-glycoprotein I (aβ_2GPI) are less commonly associated with clinical manifestations, compared to the lupus anticoagulant (LA) test [20, 21].
- Representation of potentially important new aPL tests, for instance, antibodies directed against domain I of β_2GPI or anti-phosphatidylserine/prothrombin antibodies that may be superior in predicting thrombosis [22, 23].

There have been important advances in the methodology of classification criteria development; specifically, a number of biases related to selection of cases and controls challenge older classification criteria for other rheumatic diseases [24]. The American College of Rheumatology (ACR) has published new recommendations for development and validation of criteria sets, based on contemporary standards of measurement [25, 26]. Although widely accepted, the measurement properties of the Revised Sapporo APS Classification Criteria, then not formally compared, are unlikely to meet recommended standards of measurement [27].

Finally, the task force chairs designed a needs assessment survey [28]; 92% of participants reported the need for new APS Classification Criteria.

Methods to Develop New Antiphospholipid Syndrome Classification Criteria

Our approach to develop new classification criteria for APS, endorsed by ACR and European League Against Rheumatism (EULAR) (further discussed below), employs expert-based and data-driven methods. For each phase, we use bias reduction strategies; experienced physicians in APS and patient cohort data are included. The approach involves the following four phases:

Phase I: Item generation (completed) identifies a comprehensive list of candidate criteria that classify patients according to their likelihood of having APS. International physician-scientists, selected based on their clinical and/or research interest in APS (our master list), were asked to list all features that, in their experience, occur as part of aPL/APS spectrum. In addition, we generated an extensive list of potential aPL manifestations, in particular those occurring at the time of APS diagnosis, based on our literature review.

Phase II: Item reduction (ongoing) reduces the list of candidate criteria generated in phase I to a manageable number, with the guidance of systematic reviews [17, 19, 22, 29–32] and meta-analyses. This phase uses a three-round Delphi exercise and/or nominal group technique (NGT). Physician-scientists from our master list will be asked to rank the candidate criteria based on the level of appropriateness for classification of patients with a high likelihood of APS. The criteria remaining after phase II should demonstrate good face, discriminant, and construct validity; items with very low sensitivity or specificity, poor reliability, or insufficient feasibility will be removed. Additionally, systematic reviews and meta-analyses will help experts identify those items generated in phase I that are associated with APS [20, 21, 33–35].

Phase III: Further item reduction, weighting of items, and initial threshold identification with the goal of further reducing the number of candidate criteria. Determining the relative weight of each of the criterion, and identifying a threshold for "high likelihood of APS" using multicriteria decision analysis. An APS expert core group will be created from selected task force members and physician-scientists of the master list. A face-to-face meeting will be conducted to grade APS case scenarios reflecting a broad spectrum of aPL manifestations; participants will rank-order case vignettes from lowest to highest likelihood of having APS. Multicriteria decision analysis is a methodological approach that demonstrates validity [36, 37]; the 1000Minds software will provide a systematic way to determine the relative weights of each criterion through the use of forced-choice methodology [36], allowing us to determine a hierarchy among a reduced number of criteria important in APS classification and derive a preliminary new APS Classification Criteria document. Higher scores will be expected to correlate with a higher probability that the experts would classify the case as APS. Using these results, an initial threshold score will be identified to determine the likelihood of having APS for patients presenting with aPL-related laboratory and clinical manifestations.

Phase IV: Refinement and validation; using the derivation cohort (cases and controls), the operating characteristics of the new classification criteria will be iteratively tested and refined, to achieve the fewest criteria (redundant and low-frequency criteria will be removed) and a simplified weighting system with excellent performance characteristics. Then the sensitivity, specificity, and exact binomial confidence intervals of the final APS Classification Criteria will be compared to clinical expert diagnosis and to the Revised Sapporo APS Classification Criteria [1, 3], using a new validation cohort that includes aPL-positive patients with or without SLE.

Accomplishments of the Task Force on Antiphospholipid Syndrome Classification Criteria (2013–2016)

Creation of the Task Force

The International Congress on aPL takes place every 3 years. In preparation for the 14th International Congress on aPL (September 2013, Rio de Janeiro, Brazil) (Chair, Dr. Roger A. Levy), several task forces reviewed and discussed the controversial aspects of APS in an evidence-based manner: laboratory diagnostics and trends (Chair, Dr. Laura Bertolaccini) [22], obstetric diagnostics and treatment (Chairs, Drs. Guillherme de Jesus and Ware Branch) [19], clinical diagnostics (Drs. Mirhelen de Abreu and Roger Levy) [17], treatment trends (Chair, Dr. Doruk Erkan) [33], and catastrophic APS (Chair, Dr. Ricard Cervera) [32]. In preparation for the 15th International Congress on aPL (Istanbul, Turkey, relocated to North Cyprus, September 2016), the Scientific Planning Committee merged the first three task forces under Task Force on APS Classification. Scientific Planning Committee members with relevant experience and interested in participating in the task force were also included. Dr. Karen Costenbader, who has experience in developing classification criteria, chairs this task force, together with Drs. Doruk Erkan, Stephane Zuily, and Medha Barbhaiya. Dr. Francis Guillemin, past chairman of the EULAR Standing Committee of Epidemiology and Health Service Research, participates in the task force. Dr. Ray Naden, who has led past ACR/EULAR classification criteria development initiative and is an expert in multicriteria decision analysis and 1000Minds software, participates as a consultant (Table 15.1).

Needs Assessment Survey

The task force chairs designed a 14-question needs assessment survey, emailed to 13 members in August 2014. The survey response rate was 100%; responses were analyzed anonymously in a descriptive fashion that revealed consensus

Table 15.1 Task force for the classification of antiphospholipid syndrome (APS)

Member	Affiliation	Specialty and APS expertise
North America[a]		
Mary-Carmen Amigo	ABC Medical Center, Mexico City, Mexico	Rheumatology Damage/risk assessment, cardiac APS
Medha Barbhaiya	Brigham and Women's Hospital, Boston, USA	Rheumatology, epidemiology Database management
Ware Branch	University of Utah, Salt Lake City, USA	Maternal and fetal medicine Obstetric APS
Karen Costenbader	Brigham and Women's Hospital, Boston, USA	Rheumatology, epidemiology Classification criteria development
Michael D. Lockshin	Hospital For Special Surgery, New York, USA	Rheumatology Definition, treatment, obstetric APS
Ray Naden	McMaster University, Hamilton, Ontario, Canada	Obstetrical Medicine 1000Minds software, classification criteria development
Rohan Willis	University of Texas, Galveston, USA	Immunology aPL assays, mechanisms
Europe[b]		
Tadej Avcin	Ljubljana University Medical Centre, Slovenia	Pediatric rheumatology Pediatric APS, database management
Maria Laura Bertolaccini, MD	St Thomas Hospital, London, United Kingdom	Immunology aPL assays, mechanisms
Philip G. de Groot	University Medical Centre, Utrecht, Netherlands	Biochemistry Mechanisms, aPL-related proteins
Francis Guillemin	Lorraine University, Nancy, France	Rheumatology, epidemiology Outcome measurement
Maria Tektonidou	University of Athens, Athens, Greece	Rheumatology Renal APS
Denis Wahl	Lorraine University, Nancy, France	Vascular medicine, epidemiology Meta-analysis
South America		
Mirhelen de Abreu	Federal University, Rio de Janeiro, Brazil	Rheumatology, epidemiology Decision-making
Guilherme de Jesus	State University, Rio de Janeiro, Brazil	Maternal and fetal medicine Obstetric APS
Roger Levy	State University, Rio de Janeiro, Brazil	Rheumatology Treatment, SLE-associated APS

[a]Regional Chair: Doruk Erkan
[b]Regional Chair: Stephane Zuily

regarding the need for new APS Classification Criteria [28]. Ninety-two percent of those queried reported the need for new APS Classification Criteria; 100% agreed that all disease domains are not sampled by current criteria; 85% reported scenarios in which current criteria disagree with expert diagnoses, for instance, "non-criteria" manifestations only with or without aPL, non-criteria obstetrical findings with aPL, and high suspicion for APS in patients without persistent or low titer aPL; and 62% agreed that other aPL tests should be considered. Thus, the task force decided to proceed with efforts to prepare new classification criteria.

Meta-Analysis of Different Antiphospholipid Antibody-Related Manifestations

Under the leadership of Dr. Stephane Zuily, four different teams have been working on the meta-analyses of the selected non-criteria manifestations of aPL, i.e., livedo reticularis, thrombocytopenia, hemolytic anemia, and aPL nephropathy [38–41]. These four meta-analyses, together with others [33–35, 42–45], will guide physician-scientists during the new classification criteria development efforts.

Special Sessions Organized by the Task Force

During the 2014–2016 ACR Annual Scientific Meetings, as part of a study group, our classification criteria update efforts have been presented to other task force members, APS researchers, and interested physicians. These sessions share our plans with others and demonstrate inclusiveness. The results of the needs assessment survey were presented at the ACR Annual Scientific Meeting and discussed in detail with worldwide physicians interested in APS. During the 15th International Congress on aPL (www.apsistanbul2016.org), the task force organized a session together with the Scientific Planning Committee of the congress; discussions included the historical aspects, limitations, and strengths of the Sapporo Classification Criteria, the methodology to develop classification criteria, and the accomplishments and future plans of the task force.

Completion of Phase I Item Generation

During phase I, our goal was to identify candidate criteria for the new classification criteria [46–48]. Fifty-four physician-scientists from our master list were asked three questions via e-mail:

1. "Describe all features (historical, clinical, laboratory, radiological, and pathological) that, in your experience, occur as part of the aPL/APS spectrum." This question allowed us to identify candidate criteria with potential positive weight.
2. "Describe all features (historical, clinical, laboratory, radiological, and pathological) or concomitant diseases that, if present, would make you question the diagnosis of APS even if aPL tests are positive." This question allowed us to identify criteria with potential negative weight.
3. "When you consider the diagnosis of APS, do you think of APS patients in different subpopulations?" This question allowed us to group the criteria.

We encouraged respondents to consider their real-life experiences with aPL-positive patients, rather than focusing on current APS Classification Criteria. Responses were systematically clustered by the task force members by organ system to avoid duplication and for ease of interpretability.

The phase I response rate was 76% (41/54 respondents), of whom 18 were rheumatologists, five clinical immunologists, five hematologists, five nephrologists/cardiologists/neurologists, four internists, two pediatric rheumatologists, and two obstetricians. One hundred and fifty-two candidate criteria, displayed by organ systems, were generated (Table 15.2, laboratory variables and family history are also included). The distribution of non-obstetrical candidate criteria with potential negative weight generated is shown in Table 15.3. Additional obstetrical candidate criteria with potential negative weight were late reproductive age, early/very early

Table 15.2 Distribution of 152 candidate criteria by organ system

Organ system	Number of variables[a]
Neurologic	25 (4)
Laboratory (aPL)	23 (12)
Obstetric	16 (4)
Dermatologic	15
Renal	12 (2)
Vascular	10 (5)
Cardiac	9 (2)
Laboratory (non-aPL)	9
Other	7
Hematologic	5 (2)
Pulmonary	5 (1)
Gastrointestinal	4
Musculoskeletal	4
Endocrinologic	3
Ophthalmologic	2
Auditory	2
Family history	1 (1)

[a]Number of variables with subcategories is indicated in parentheses

Table 15.3 Distribution of candidate criteria with potential negative weight (clustered) (number of responders in parenthesis)

Candidate criteria clustered	Selected responses
Traditional CVD risk factors (*n*:12)	Significant atherosclerosis
Infections (*n*:15)	HIV, HCV, HBV, Lyme, syphilis, rheumatic fever, mycobacterium, Ebstein-Barr Virus
Laboratory (aPL) (*n*:18)	Low titer ELISA, single positive, isolated IgM, fluctuating titers, non-criteria tests
Other thrombosis risk factors (*n*:14)	Surgery, immobilization, injury, atrial fibrillation, PFO, infective endocarditis, oral contraceptives, genetic thrombophilia, nephrotic syndrome
Malignancy (*n*:11)	Hematologic, atrial tumor
Autoimmune disease (*n*:10)	SLE, RA, vasculitis, Sjogren syndrome, overlap syndrome, thyroid disease, Behcet, Takayasu
Histological (*n*:7)	LCV, fibrinoid necrosis, GN, inflammation, no thrombosis, normal placental histology
Elderly (*n*:7)	>50 years old, >55 years old, >60 years old, >70 years old
Neurologic disease/symptoms (*n*:5)	Multiple sclerosis, epilepsy, chronic headache
Thrombotic microangiopathies (*n*:7)	HUS, DIC, TTP, PNH, heparin-induced, sepsis
Laboratory (non-aPL) (*n*:7)	High ESR/CRP, severe thrombocytopenia, high titer lupus serology
Radiological (*n*:6)	No vasculopathy, thrombosis, or vessel wall thickness, demyelinating lesions, interstitial lung disease
Medications (*n*:6)	Anti-Tumor Necrosis Factor use
Lupus manifestations (*n*:3)	Pericarditis, arthritis, malar rash, discoid rash
Strong family history (*n*:1)	Thrombosis, recurrent miscarriages
Bleeding (*n*:1)	Spontaneous with platelet > 50×10^9/L
Other (*n*:1)	Degos disease, Devic disease, thromboangiitis obliterans

CVD cardiovascular disease, *GN* glomerulonephritis, *HIV* human immunodeficiency virus, *HCV* hepatitis C virus, *HBV* hepatitis B virus, *OC* oral contraceptive, *SLE* systemic lupus erythematosus, *RA* rheumatoid arthritis, *PFO* patent foramen ovale, *LCV* leukocytoclastic vasculitis, *HUS* hemolytic uremic syndrome, *DIC* disseminated intravascular coagulopathy, *TTP* thrombotic thrombocytopenic purpura, *PNH* paroxysmal nocturnal hemoglobinuria

(recurrent) miscarriage, normal placental pathology, and concomitant causes of recurrent miscarriage, such as uterine/cervical abnormality, thyroid diseases, or genetic abnormalities. The reported subpopulations of APS patients, grouped based on age, clinical manifestations, aPL profile, and risk level, are shown in Table 15.4.

The phase I item generation step of our new APS Classification Criteria supports the concept that clinical manifestations of aPL are heterogeneous and complex and that the illness may be multifactorial. We confirmed that physicians think of patients with aPL in different subpopulations.

Table 15.4 Reported subpopulations of antiphospholipid syndrome patients

Age	Young vs elderly Neonatal vs pediatric vs adult
Clinical manifestations	Yes vs no
	Possible vs definite
	Systemic vs nonsystemic
	Criteria vs non-criteria
	Associated with other autoimmune diseases vs not
	Associated with other infection/malignancy/drugs vs not
	Catastrophic vs non-catastrophic
	Thrombotic vs non-thrombotic
	Venous thrombotic vs arterial thrombotic
	Microvascular/microangiopathic vs not
	Obstetric vs thrombotic (non-obstetric)
	Early obstetric vs late obstetric
	Hematological vs not
	Neurologic/cardiac vs not
	Refractory to conventional treatment vs not
aPL profile	Seronegative vs seropositive
	Triple vs double vs single positivity
	Triple vs LA positivity vs others
	LA positivity vs aCL positivity
	Persistent vs not
Risk level	"High risk" vs "medium risk" vs "low risk

Group Conclusion and Future Plans

Employing methodology used for the development of classification criteria for other autoimmune diseases [36, 49–50], the new APS Classification Criteria should have excellent face validity, criterion validity, and performance. The task force has completed phase I item generation and will proceed to phase II item reduction.

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Chapter 16
15th International Congress on Antiphospholipid Antibodies Task Force on Pediatric Antiphospholipid Syndrome Report

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Introduction

The antiphospholipid syndrome (APS) is an autoimmune multisystem disease characterized by thromboembolic events, pregnancy morbidity, and hematological, dermatological, neurological, and other manifestations in the presence of elevated titers of antiphospholipid antibodies (aPL) [1]. Antiphospholipid syndrome may occur as an isolated clinical entity (primary APS) or in association with other diseases, mainly systemic lupus erythematosus (SLE). It occasionally occurs with other autoimmune conditions, infections, and malignancies. Pediatric APS due to de novo production of aPL occurs anytime from the neonatal period through childhood and adolescence. Neonatal APS, manifesting with thrombotic events, is a rare complication; it may be associated with maternal aPL, in which case it is attributed to transplacental passage of aPL, or it may be found in newborns whose mothers do not have aPL. The rapid development of multiple organ thromboses and microthromboses, known as catastrophic APS (CAPS), has been reported in children [2].

In recognition of the unique issues surrounding the diagnosis and treatment of pediatric APS patients, a pediatric APS task force was developed in preparation for the 15th International Congress on aPL (www.apsistanbul2016.org) (North Cyprus, September 2016). This multidisciplinary group consists of pediatric rheumatologists, pediatric neurologists and neurodevelopment specialists, pediatric hematology-coagulation specialists, and neonatal-perinatal medicine specialists. The objectives of this task force were to review the current knowledge on the pathogenesis, clinical and laboratory features, diagnosis, classification, and treatment of the pediatric APS to generate recommendations for future research and to suggest modifications to the APS classification criteria for enhanced applicability to children and neonates. Members of this task force have published two extensive reviews of the literature [3, 4].

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A Brief Review of Hemostasis

Hemostasis, the normal reparative process for damaged vasculature, is a three-stage process (vessel damage and platelet response, thrombin generation, and fibrinolysis) with the following participants: cells or cellular remnants (platelets, monocytes, endothelial cells, and microparticles), proteins (hemostatic and fibrinolytic), and vascular endothelium, together known as the cell-based model of hemostasis [5–7].

Inhibitory mechanisms that modulate hemostasis include blood proteins (proteins C and S, antithrombin [AT], α_2-macroglobulin, and tissue factor pathway inhibitor [TFPI]); vessel wall proteins (thrombomodulin, endothelial protein C receptor, and TFPI) or chemicals (nitrous oxide [NO], CD39 [ectonucleotidase]); prostacyclin; and proteoglycan-related glycosaminoglycan molecules (chondroitin, dermatan sulfate, or heparin sulfate) [8, 9].

Vessel Damage and Platelet Response (Adhesion, Activation, and Aggregation)

The first stage of hemostasis is initiated following blood vessel damage as a result of vessel severing, for instance, surgery, trauma, or clinical conditions like cancer [7]. Von Willebrand factor (vWF) adheres to the exposed collagen within the damaged vessel wall and attracts platelets through receptor glycoprotein (GP)-Ib-IX-V [10]. This binding is not firm, but it causes platelet GPIIb-IIIa receptor (outside-in signaling) to become active and bind to vWF. Platelets adhere to the area of damage, express negatively charged phospholipids (primarily phosphatidylserine) [11], become activated, and release granule contents. Alpha granules secrete hemostatic factors (factor V [FV], vWF, fibrinogen), angiogenic factors (angiogen, vascular endothelial growth factor [VEGF]), anti-angiogenic factors (anti-angiostatin, platelet factor 4), growth factors (platelet-derived growth factor [PDGF], basic fibroblast growth factor [bFGF], stromal cell-derived factor 1 [SDF1α]), proteases (matrix metallopeptidase 2 [MMP2], matrix metallopeptidase 9 [MMP9]), necrosis factors (tissue necrosis factor α [TNFα], tissue necrosis factor β [TNFβ]), and other cytokines. Dense granules secrete the small molecules serotonin, adenosine diphosphate (ADP), and polyphosphates [12]. Many of the components promote further platelet activation. Fibrinogen binds to the platelet receptor, GPIIb-IIIa, and, through multiple receptor binding sites, binds activated platelets to form an aggregated mass [13]. Inhibitory mechanisms of platelet aggregation within the endothelium prevent propagation of platelet aggregation beyond the area of vascular injury; these mechanisms include endothelial NO, prostacyclin, and ectonucleotidase [7, 10].

Thrombin Generation in the Cell-Based Model of Coagulation

The second stage of hemostasis occurs following activation of coagulation through factor VII activation by tissue factor (TF), which is released from damaged vascular endothelium and is expressed by monocytes, macrophages, neutrophils, activated endothelial cells, platelet microparticles, and smooth muscle [6, 7]. Factor X is activated by VIIa-Va-TF complex and combines with FVa activating FII (prothrombin), resulting in a small amount of thrombin (IIa) production [13]. Thrombin causes activation of platelets, which is demonstrated by expression of negatively charged phospholipids on their surfaces, release and activation of FV from the dense granules, and activation of FXIII. In addition, thrombin releases FVIIIa from vWF, which is also expressed on the platelet surface. FIXa and FVIIIa complex on the negatively charged phospholipids of the activated platelet surface and activate FXa. The activated platelets expressing the complex FXa-Va then act on FII producing thrombin, resulting in fibrin monomer production, which is polymerized by factor XIII [5, 6, 13].

Modulation of coagulation is carried out by antithrombin (AT), which inhibits FXa, FIIa, and TFPI, which then inhibits TF-FVIIa complex, FXa, and protein C, which itself is activated by thrombin when it binds to the membrane receptor, thrombomodulin, and endothelial cell protein C receptor. This complex subsequently binds to protein S and inhibits FVa and FVIIIa [8].

Fibrinolysis (Clot Dissolution) and Modulation

The third stage of hemostasis, fibrinolysis, involves dissolution of the fibrin clot. Production of thrombin activates tissue plasminogen activator (tPA), the most important activator of plasminogen, which is subsequently converted to plasmin. Plasmin degrades the polymerized fibrin clot into small fragments known as d-dimers [14]. Modulation of fibrinolysis is carried out by decreasing plasminogen availability, either through degradation by thrombin-activatable fibrinolysis inhibitor (TAFI) or inhibition by plasminogen activator inhibitor (PAI) [14]. In addition, α_2-macroglobulin inhibits plasmin activity [14].

Hemostasis Measurement

Hemostasis can be measured by determining the concentration or amount of participants (coagulation and fibrinolytic factors, platelets), interaction of the participants in a plasma-based system (activated partial thromboplastin time [aPTT], prothrombin time [PT] converted to the international normalized ratio [INR], thrombin

clotting time [TCT], and platelet aggregometry) or in whole blood (d-dimer, activated clotting time [ACT], viscoelastic testing of clot formation, i.e., thromboelastography or thromboelastometry). The plasma-based assays have been criticized for not reflecting in vivo hemostasis due to the absence of cellular components, which are major participants in the process. An overview of hemostatic testing can be found elsewhere [5, 14, 15].

Developmental Hemostasis: Differences Between Children and Adults

Children differ from adults in the participants and the inhibitory mechanisms of hemostasis, thereby influencing the epidemiology, clinical manifestations, and management of thrombosis.

Platelets

Platelets in neonates and children are present in the same number and size, with the same receptors [16–18]. (The cited papers do not compare neonates or children to adults.) However, depending on the method of testing, neonatal platelet function may be decreased or increased. Neonatal platelets have *decreased* function when measured by granule secretion [19, 20]; aggregometry using the agonists epinephrine, collagen, ADP, and thromboxane [21]; and activation markers P selectin and CD63 [19, 20, 22]. These differences in function are due to decreased numbers of fibrinogen binding sites exposed on GPIIb-IIIa, impaired receptor-mediated signal transduction at thromboxane receptor [23], impairment of calcium mobilization [24], and decreased adrenergic receptors (epinephrine) [25]. Neonatal platelets have *increased* function when demonstrated by whole blood assays measuring platelet function by PFA 100 [26] and adhesiveness/aggregation on extracellular matrix-coated cone and plate analyzer [18]. These results suggest enhanced platelet and vessel wall interaction, perhaps maximized by other neonatal differences in whole blood composition, for instance, increased hematocrit, red cell volume, vWF concentration, and large-molecular-weight forms [26–28].

Coagulation Proteins

The integral proteins in the hemostatic system (factors XII, XI, X, IX, VIII, VII, V, II, and fibrinogen) are synthesized by the fetus [29, 30]. Levels of the proteins approach adult levels at different times during childhood; the contact factors, FXII and FXI, and vitamin K-dependent factors (FII, FVII, FIX, FX) reach adult levels

by about age 5 [31]. In newborns, fibrinogen levels are quantitatively the same as adults, but the protein is qualitatively different, with increased glycosylation (sialic acid) and phosphorus content and decreased rate of polymerization with the ratio of function to quantity being increased compared to children and adults [32]. Inhibitory proteins, proteins C, S, and AT, are decreased and approach adult levels at 3–6 months of age [33]. α_2-Macroglobulin levels are increased until adolescence when they approach adult levels [33]. The amount of thrombin produced is decreased in neonates and children [30, 34]. However, despite the reduced thrombin generation demonstrated in infants and neonates, the levels of coagulation inhibitors are normally less than in adults, and thus the lag time to thrombin generation is much shorter [35, 36]. Tissue factor pathway inhibitor (free form) is decreased throughout childhood compared to adults [30].

Fibrinolysis

Regarding fibrinolysis, children ages 1–18 years and adults have similar levels of tPA, PAI, and TAFI [37]. Compared to adults, plasminogen levels are decreased until 6 months of age, with slow activation kinetics by tPA [31]. Using venous occlusion technique, adolescents demonstrate decreased levels of tPA antigen and increased activity of PAI-1 resulting in ex vivo increased clot lysis times [38], suggesting decreased fibrinolysis in children. Other laboratory techniques suggest increased fibrinolysis, including increased d-dimer levels in neonates and children, and by functional fibrinolysis testing in neonates using thromboelastometry [30, 39, 40].

Vascular Endothelium

Studies in rabbits document structural and functional differences between prepubertal and adult animals in the aortic and inferior vena cava endothelium [41, 42], specifically in endothelial function (increased inhibition of thrombin activity), extracellular matrix structure, proteoglycan distribution (increased chondroitin and heparin sulfate), glycosaminoglycan content (increased chondroitin and dermatan sulfate), and function [41, 42]. The endothelium of newborn mice phenotypically resembles dysfunctional endothelial cells of vascular disease, with prominent stress fibers and marked inhibition of endothelial dilation [43].

Differences in Hemostatic Testing in Children

Differences in components of hemostasis in children result in differences in hemostatic testing. The aPTT and PT/INR are prolonged in newborns up to late adolescence and remain slightly higher but significantly elevated compared to adults [30].

The TCT is increased but approaches adult levels by 6 years of age [30]. Global hemostasis testing using either thromboelastography or thromboelastometry in neonates and children demonstrates faster initiation of coagulation despite the increased aPTT/PT, but with varying results between studies for clot firmness [28, 44–48]. The plasma-based assays have been criticized for not reflecting in vivo hemostasis due to the absence of cellular components. An overview of hemostatic testing can be found elsewhere [5, 14, 15].

The differences in hemostasis add additional confounding variables to the epidemiology and management of children with or at risk for thrombosis. Hemostatic testing results are often further altered when aPL increases aPTT and/or PT/INR. Children with APS are at increased risk for thrombosis [49–51]; whether the risk is greater than adults is unknown [52–54].

Thrombosis Risk Assessment (Genetic and Extrinsic)

Assessment of Virchow's triad of stasis, vascular injury, and hypercoagulability is inherent in the assignment of thrombotic risk. The antithrombotic milieu in a child slowly acquires characteristics of an adult with the onset of puberty. Compared to adults, children are relatively protected from thrombosis because of the decreased potential for thrombin generation, increased α_2-macroglobulin, and the antithrombotic potential of the vascular wall. Children may acquire additional risk factors in adolescence, including smoking, birth control pills, and pregnancy. Preexisting risk factors such as obesity and dyslipoproteinemia, which may have been present earlier, have an amplified effect. As the vascular endothelium decreases in antithrombotic potential, vascular injury from play activity and sports may impose additional risk.

Antiphospholipid antibodies coincident with inherited thrombophilia (for isolated or combinations of genetic mutations) are associated with increased relative risk of thrombosis. The increased risk of thrombosis associated with aPL and factor V Leiden mutations in adults and children has been discussed elsewhere [55–58].

Clinical and Laboratory Features of Antiphospholipid Syndrome in Children

Antiphospholipid syndrome plays an important role in pediatric venous and arterial thromboembolic events, including stroke [59, 60], but is rare among all patients diagnosed as having APS: onset prior to the age of 15 occurred in only 2.8% of all patients with APS [61]. Pediatric and neonatal APS have been addressed by few studies [62–64].

Although the criteria of obstetric morbidity are not applicable to prepubertal children, and despite lack of validation in children and neonates, the same diagnos-

tic criteria are used for classification of pediatric APS [65]. Nonetheless, pediatric APS presents with symptoms similar to adults [66]. Katzav et al. [67] showed that patients with childhood-onset APS presented with more episodes of chorea and jugular vein thrombosis than did adults [67]. Arterial thrombosis is more common in children [63, 68]. In a cohort of 28 children with APS [69], the most common initial manifestations were venous thrombosis, stroke, and thrombocytopenia. Lupus anticoagulant was detected in 96% of those tested. Seven of the 24 patients with vascular thrombotic events had recurrences. Hereditary thrombophilia was more common in children who experienced a single episode of APS (8 [53.3%] of 15 patients) than in those who experienced recurrences (2 [28.6%] of 7 patients). However, only two patients in the latter group (28.6%) received anticoagulants after the first manifestation, compared with 12 (70.6%) of the 17 patients without recurrences. Infants with perinatal stroke usually display a monophasic disease, and other manifestations of APS do not develop later.

Based on very small studies, levels of IgA anticardiolipin (aCL) antibody are lower in children with APS than in adults, whereas IgG anti-β_2-glycoprotein-I (aβ_2GPI) levels are highest in preschool children [69, 70].

An international registry of pediatric APS, established in 2004 [63], currently documents standardized clinical, laboratory, and therapeutic data of 140 children with definite APS seen at 24 pediatric university centers. Thrombotic events include venous thrombosis in 60%, arterial thrombosis in 32%, small vessel thrombosis in 6%, and mixed arterial and venous thrombosis in 2% of the first 121 patients [63]. During a follow-up period of 6.1 years, 19% developed recurrent thrombosis [63] and 5% developed catastrophic APS.

Perinatal stroke is a special entity in children with APS [71, 72]. Perinatal arterial stroke (PAS) may result from focal arterial or venous thrombosis or emboli occurring between 20 weeks of fetal life and the 28th postnatal day. Diagnosis may be delayed; reported prevalence is 1:4000–5000 live births [72]. Risk factors may relate to both fetal and neonatal disorders as well as maternal and placental conditions. The relative roles of genetic and acquired thrombophilia in the pathogenesis of PAS are controversial. Positive aPL tests can be found in both neonates with PAS or their mothers, or both [60]. Berkun et al. [72] presented a cohort of 12 neonates with cerebral thromboembolism and persistent aPL whom they followed prospectively with repeated antibody testing. In 10/12 cases aPL levels decreased to normal within a median of 2.5 years. Notably, following the diagnosis of perinatal cerebral event, 11 of the patients received no anticoagulant therapy; a single infant who suffered from perinatal cerebral sinus venous thrombosis was treated with low-molecular-weight heparin (LMWH) until the age of 6 months. In this cohort, maternal aPL was detected in only two cases, not necessarily correlating with the antibodies found among the affected infants. Similarly, within an Israeli PAS cohort of eight mother-infant pairs [60], aPL discordance was noted between mothers and their offspring: in three cases, only the mother had aPL; in four, only the infant; and in one, both mother and infant. These findings indicate that maternal antibodies are not the only pathogenic factor for neonatal APS. An important finding was the absence of recurrent thrombosis or other APS manifestations, despite lack of prolonged anticoagulation; also, there was gradual disappearance of aPL. Timing

of perinatal brain injury introduces multiple, often competing, factors in brain maturation and development that will ultimately determine outcome.

Epidemiologic pediatric studies show that, from 1 year to puberty, pediatric patients are less prone to thrombosis than are adults. Low titer and transient aPL [72] are frequent within the pediatric population, but thrombosis is rare. Overall, perinatal stroke in children with aPL may not require anticoagulant therapy unless other risk factors prevail.

A few registry-based studies suggest that neurological manifestations occur in 16–22% of pediatric APS cases [63, 73]. They include thrombosis (arterial and venous); immune-mediated inflammatory change; and secondary complications or symptoms, for instance, seizures, headaches, focal neurological deficits/signs, and cognitive changes. Psychiatric symptoms, including psychosis and mood disturbance, have also been described [63, 74].

Regarding lupus-associated APS, the most common neurological manifestation is thrombosis, including both arterial stroke and venous sinus thrombosis [74]. The 121-patient pediatric APS registry found a 16% prevalence of headache, 9% of cognitive dysfunction, and 10% of psychosis [75]. Comparison of lupus patients with and without aPL suggests an association between aβ_2GPI and neuropsychiatric manifestations ($p = 0.02$). Among 32 patients with both primary and other autoimmune disease-associated aPL, and that defined neurological manifestations only as epilepsy, chorea, or aseptic meningitis, seizures were the most common neurological manifestation, occurring in five (16%) [73].

In studies documenting the rate of aPL positivity in neurologic cohorts, there is no increase of aPL in pediatric headache patients compared to controls [76]. Approximately 30% of 142 consecutive pediatric epilepsy patients in one cross-sectional cohort study were positive for aPL, but selection bias and lack of controls in this study limit generalizability of the findings [77]. In another study of 80 pediatric epilepsy patients, only three (3.8%) were positive for aPL, with aCL found in one (1.3%) [78].

In pediatric APS, neurological complications are common and may be significant. Future studies should provide detailed documentation of functional and structural neurological changes, including detailed cognitive and neurological assessments and MRI studies using advanced metrics.

Prevention and Treatment of Antiphospholipid Syndrome in Children

Assessment of the risk posed by acquired or extrinsic factors is essential to treatment decisions [79, 80]. The treatment of "at-risk" children may not always include anticoagulants but may include dietary changes, behavior modification, and/or treatment of associated conditions (none of which, however, are evidence-based). Endothelial activation and endothelial damage associated with inflammation may shift the balance toward thrombosis. Thus, treatment of an underlying disease may be of benefit. In an otherwise-healthy, low-risk child with a postinfectious event, this prothrombotic

situation may be transient; however, in children with underlying rheumatic diseases, anticoagulation therapy may need to be continued until disease activity is quiescent. Deitcher and Carman [81], studying adults, suggested that "lifelong" and "long-term" treatment are different, the latter implying that the issue will be revisited. Risk stratification and individualized therapy determine how long a patient is treated.

In children, identification of risk factors and provision of preventive health, and short-term treatment of transient events, may be more important than in adults because of developmental hemostasis [82].

A recent European project, SHARE (*S*ingle *H*ub and *A*ccess Point for Pediatric *R*heumatology in *E*urope), systematically evaluated published data on APS in pediatric populations to provide 14 evidence-based recommendations for the diagnosis and treatment. When available, these recommendations will help practicing physicians and facilitate improvement and uniformity of care of children with APS [83].

Long-Term Follow-Up of Children Born to Mothers with Antiphospholipid Antibodies

Neonatal Thrombosis in Newborns of Antiphospholipid Syndrome Mothers

In 71 live newborns from mothers with primary APS who were compared with equal number of healthy mothers' live newborns retrospectively matched for gestational age, birth weight, mode of delivery, and obstetrical complications, there were no differences in neonatal intensive care unit admissions and organ complications [84]. (Eighteen fetuses [20%] of APS patients did not survive.) Reports of thrombosis in offspring of APS patients are rare: 15 reports produced 24 cases [85]; in the European Registry, of 134 children from 133 APS mothers (81% primary APS) followed up at 5 years, none had thrombosis [86–88]. Of 201 pregnancies in 125 primary APS patients followed in a single center from 1985 to 2014, there was one case of neonatal APS (1110 g at 31 weeks of gestation). Thus, aPL-related thrombotic complications in neonates are rare, despite the potential pathogenic of transplacentally transmitted IgG aPL [89–91].

Neuropsychological Development of Children Born to Mothers with Positive Antiphospholipid Antibodies (With/Without Lupus)

Maternal lupus does not seem to impair the intelligence of their children, even though early studies reported a tendency to learning disabilities in males [92–94]. In a study of 19 children of SLE patients (mean age 10 years; range 6–16 years), two had learning problems, and one was diagnosed as a "bad reader," all three born to

aCL or LA positive mothers. Among 11 children without learning problems, five were born to aCL or LA positive mothers, suggesting that learning disabilities in children of lupus women may be related to maternal aPL [95]. To test this hypothesis, 17 children from primary APS mothers (mean age 11.4 years) were studied with multiple neuropsychological tests [96] and compared with 12 children with rheumatoid arthritis (mean age 12.6 years) [97]. Language delay and learning disabilities were more frequently detected in children of APS mothers, at a higher rate than the general school-age population. In the European Registry, of 134 children from 133 APS mothers (81% with primary APS), only four (3%) children had behavioral abnormalities. (In none of these studies were children matched for birth weight or for gestational age.)

The relationship between maternal autoimmune diseases and autism spectrum disorders in the offspring is also not clear. Autism belongs to a heterogeneous group of disorders with a complex multifactorial genesis [86–88]. Thirty-eight women positive for aPL (17 primary APS and 21 with other autoimmune diseases) had 39 children in whom psychomotor development appeared to be normal during the first 6 months of life [98]. In a study of 26 children (selected by unstated criteria from 233 SLE pregnancies), children were interviewed at ages categorized as under or greater than 4 years; all had normal intelligence, and intelligence was not related to maternal aPL [99]. In a different study of 60 children born of 38 SLE patients, five of nine children born to aPL-positive women were referred for special educational services for speech delay and attention-deficit disorders; the frequency of referral was higher than expected even after bivariate adjustment for birth weight and lupus anticoagulant [100].

In a recent study of 40 children (median age 7.4 years) born to mothers with SLE and/or APS carrying IgG aPL during the third trimester of gestation, the intelligence level was normal, but four children had epilepsy; sleep disorders and minor emotional/behavioral problems were found in 12 (30%) and 20 (50%), respectively. No associations were found between these problems and premature birth, SLE, or autoantibody profile [101].

The few studies on neurodevelopmental outcome of children born to mothers with APS show controversial results. However, long-term observation is reassuring regarding normal intelligence and neurological examination. In children of mothers with APS, language delay was noted, and learning disabilities are described at higher rates than a general school-age population. Additional factors, such as coping with maternal chronic disease and the impact of maternal diseases on the family and social environment, may affect neuropsychological development [97]. In addition, most studies have not been controlled for birth weight or gestational age, which may impact neurological development independently of APS.

Group Conclusion

It is expected that large pediatric databases of national registries will provide informative data on the incidence and prevalence of aPL/APS and the risk of thrombosis for the general pediatric population. These databases should also provide data on

the prevalence of maternal or fetal aPL in relation to autism, learning disabilities, and cognitive dysfunction. Current information derived from registries of high-risk populations (limited by small sample size or heterogeneous cohorts) may be too focused and not generalizable. Large studies, on general pediatric populations, which are age-stratified (to account for developmental hemostasis and extrinsic risk factors), are needed in order to establish risk. Treatment recommendations can be proposed only after risk is identified and defined. The risk of thrombosis changes as children grow because of developmental hemostasis and changing extrinsic risk factors related to age and social pressures.

Risk stratification models are useful in the assessment of how, when, and how long to anticoagulate APS patients [102]. Large comparative studies will be needed to assess the effectiveness and safety of the new antiplatelet drugs, dual antiplatelet therapy, new oral anticoagulants/inhibitors, and parenteral anticoagulants in the pediatric APS population at risk for thrombotic events.

Now is the time to focus on new registries that target aPL-positive patients. Surveys of practice patterns may be useful, as may be the assembly of a template of cases for criteria development and validation and for testing the hypothesis that adult criteria do not work in children. The first steps have been taken; reviews of the literature and analysis of existing focused databases have been published. Suggestions have been made for the refinement of data entry questions for prospective databases, but these need further evaluation. Published proposals for revised classification criteria will need to be validated. Evaluation of therapies, especially the newer agents, still needs to be performed. Risk stratification models by patient age may prove useful and will need to be tested.

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Chapter 17
15th International Congress on Antiphospholipid Antibodies Task Force on Catastrophic Antiphospholipid Syndrome Report

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Introduction

The "Task Force on Catastrophic Antiphospholipid Syndrome (CAPS)" was originally developed at the 14th International Congress on Antiphospholipid Antibodies (aPL) in Rio de Janeiro, Brazil, in 2013. The objectives of this Task Force were to assess the current knowledge on pathogenesis, clinical and laboratory features, diagnosis and classification, precipitating factors, and treatment of this condition to address recommendations for future research. The first report of this Task Force was published in 2014 [1]. During the 15th International Congress on aPL (www.apsistanbul2016.org) in 2016, its members presented updated evidence and relevant literature in their areas of expertise. An open discussion followed to reach agreement. Where data were limited or incongruent, expert opinion supplemented the recommendations. This chapter summarizes the findings and conclusions of the Task Force.

Pathogenesis of Catastrophic Antiphospholipid Syndrome

The pathogenesis of CAPS is not fully understood. It differs from classic APS by the size of affected vessels (classic APS affects large- and medium-size vessels in most patients; these vessels are affected in only about one third of CAPS patients, in whom small-vessel thrombosis is clinically evident) and time course (isolated events in classic APS, sometimes years apart, compared to cluster of thrombotic events within hours or few days in CAPS). The frequent association of disseminated intravascular coagulation (DIC) and thrombotic microangiopathy (TMA) in CAPS also

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distinguishes between these two conditions [2]. In addition, CAPS is characterized by non-thrombotic manifestations, mainly profound systemic inflammatory response syndrome (SIRS), including acute respiratory distress syndrome (ARDS). Several theories and mechanisms have been proposed, and multiple precipitating factors have been described. It seems that a trigger (sometimes referred to as a "second hit") [3], occurring in a susceptible ("first hit") individual, is responsible for a cascade of events, resulting in extensive small-vessel thrombosis [4]. However, it is still unclear why only a few APS patients develop CAPS, while the vast majority do not.

The concept of molecular mimicry was proposed in 2000 by Asherson et al. [5]. According to this theory, homology between bacterial proteins and β_2-glycoprotein I (β_2GPI) induces anti-β_2-glycoprotein I antibodies (aβ_2GPI), which favor a hypercoagulable state. Experiments performed on murine models successfully induced autoantibodies (to β_2GPI or cardiolipin) [6–8] and thrombosis following exposure to some pathogens. This may be the "first hit" in patients who will later develop CAPS [9].

The pathophysiology of CAPS combines two major processes: small-vessel thrombosis (thrombotic microangiopathy) and SIRS. A model of the presumed pathogenesis should explain both phenomena and describe the interrelations between them. It is important to point out that none of the mechanisms described have been demonstrated in CAPS, in vitro or in vivo, and most represent extrapolation from better understood conditions, such as classic APS, thrombotic thrombocytopenic purpura, and sepsis.

Anti-β_2-GPI antibodies recognize β_2GPI peptides sharing molecular homology with some bacteria and viruses. Because microbial structures are a ligand for Toll-like receptor (TLR), it has been speculated that β_2GPI might interact with TLR, resulting in activation of endothelial cells (similar to their activation in response to microbial products, such as lipopolysaccharides). This activation induces a proinflammatory and procoagulant state.

Activation of TLR through intracellular signaling processes involving phosphorylation of IRAK (IL-1-associated kinase) results in translocation of the nuclear factor NF-kB to the nucleus in endothelial cells. This signal promotes proinflammatory state by production of cytokines (TNF-a, IL-1B, IL-6, IL-8) and procoagulant state by production of tissue factor and plasminogen activator inhibitor [2]. The aforementioned cascades are common to many etiologies for septic reaction and are not specific to aβ_2GPI. If indeed this is the pathway responsible for extensive thrombosis, then the reason for the unique manifestation of CAPS is yet to be discovered.

The complement system is presumed to have a significant role as well. Reports of successful treatment of refractory cases with eculizumab (humanized monoclonal antibody to C5) further strengthen this view [10–13].

Catastrophic APS shares many similarities with thrombotic microangiopathies (TMA), including small-vessel thrombosis, hemolytic anemia, and thrombocytopenia [2]. Schistocytes, the hallmark of microangiopathic hemolytic anemia, are reported in more than 20% of cases in the CAPS Registry [1]. Catastrophic APS was found to be the second most common form of TMA (after hemolytic uremic syndrome) in patients with aPL [14].

Recently, other mechanisms have been proposed to be involved in the pathogenesis of CAPS [15–16]. Oxidative stress was postulated to initiate thrombosis, by forming disulfide bridges and exposing major epitopes of the β_2GPI, thus "priming" the endothelium and allowing the formation of immune complexes on the cell surface [16]. Other antibodies are being investigated for their role in thrombosis in "seronegative" patients presenting with aPL-related clinical events. For example, anti-vimentin/cardiolipin complex may be responsible for clot formation by IRAK phosphorylation and NF-kB activation [12]. Interestingly, significantly higher levels of ferritin were found in patients with CAPS (compared with classic APS), and these high levels correlated with some manifestations of CAPS. It is not clear, however, if ferritin plays a role in the inflammatory cascade or is only a marker of the inflammatory response [15]. These mechanisms await further investigations to better clarify their role in CAPS pathogenesis.

Clinical and Laboratory Manifestations of Catastrophic Antiphospholipid Syndrome

As of December 2015, the CAPS Registry (http://ontocrf.costaisa.com/en/web/caps/) included 522 episodes of CAPS in 500 cases [17]: 483 patients had one episode, 12 had two episodes, and five had three episodes. Among CAPS patients, 68% were females and mean age of all patients was 38 ± 17 years. Sixty percent of patients had no other systemic autoimmune disease, 30% had systemic lupus erythematosus (SLE), 4% had clinical features of SLE but did not fulfill American College of Rheumatology SLE classification criteria, and 6% had other autoimmune disease.

Approximately, 65% of CAPS Registry cases reported a precipitating factor. The most frequent triggers were infections, accounting for up to 49% of cases, especially among the pediatric (less than 19 years old) group of patients (58%). Bacterial, viral, and parasitic infections have all been described. The most frequent infections were those affecting the respiratory tract, especially those with a suspected viral agent. Urinary tract infections and skin infections followed respiratory tract infections. Other triggers were surgical procedures and malignancies, especially in elderly patients, anticoagulation withdrawal, oral hormonal contraceptive use, pregnancy, drugs, and SLE flare.

The clinical picture of CAPS is defined by multiple organ involvement. The kidneys (73%), lungs (60%), central nervous system (56%), and heart (50%) are the most commonly involved (Table 17.1).

Thrombocytopenia is the most common laboratory feature (67%). Hemolysis is also frequently reported (37%) and is usually associated with schistocytes (22%). Some patients developed DIC (11%). Among CAPS patients, most had circulating lupus anticoagulant (LA) (83%), IgG anticardiolipin antibodies (aCL) (81%), and/or IgG aβ_2GPI (78%), while IgM aCL (49%) and IgM aβ_2GPI (40%) were less often found. Antinuclear antibodies were detected in 57% of cases and anti-double-stranded DNA antibodies in 32%, figures explained by clinical features of SLE in

Table 17.1 Clinical manifestations in 500 patients from the Catastrophic Antiphospholipid Syndrome Registry

Clinical manifestation	%
Organ involved	
Kidney	73
Renal failure	77
Proteinuria	29
Arterial hypertension	24
Hematuria	16
Lung	60
Acute respiratory distress syndrome	36
Pulmonary embolism	26
Alveolar hemorrhage	12
Pulmonary edema	8
Brain	56
Stoke	40
Encephalopathy	39
Seizures	15
Headache	8
Heart	50
Heart failure	44
Myocardial infarction	30
Valvulopathy	28
Libman-Sacks endocarditis	13
Skin	47
Livedo reticularis	43
Cutaneous necrosis	26
Cutaneous ulcers	24
Purpura	14
Liver	39
Elevated liver enzymes	63
Hepatomegaly	10
Liver failure	9
Jaundice	7
Peripheral vessel	37
Peripheral venous thrombosis	69
Peripheral arterial thrombosis	46
Gastrointestinal	24
Gastrointestinal bleeding	18
Ileus	4
Spleen	18
Adrenal glands	10

up to 40% of cases. Antibodies against the extractable nuclear antigens were less often detected: anti-RNP in 8%, anti-Sm in 5%, anti-La in 4%, and anti-Ro in 9% (percentages calculated according to the number of patients with these tests recorded).

"Thrombotic Storm" and Differential Diagnosis of Catastrophic Antiphospholipid Syndrome

Thrombotic storm refers to a clinical presentation of acute development of multiple thromboembolic events involving diverse vascular beds [18]. Affected individuals are typically young, and thromboembolic events often involve unusual sites [18, 19]. The term "thrombotic storm" was first used by Kitchens in 1998, and then the clinical phenotype was proposed by a multidisciplinary group for clinical studies. The characteristics of thrombotic storm include (a) an underlying hypercoagulable state; (b) a provocative factor or "trigger" associated with initiation; (c) new thromboembolic events developing rapidly, especially if therapy is delayed; (d) importance of prompt initiation of antithrombotic therapy ; and (e) good long-term prognosis, if the cycle of thrombosis is interrupted early. Of the six patients in Kitchen's report, three likely had CAPS [20].

In addition to CAPS, several disorders are included under the umbrella of thrombotic storm including [21] atypical presentations of thrombotic thrombocytopenic purpura (TTP), in which thrombotic occlusions may develop days to weeks before the development of thrombocytopenia and characteristic microangiopathic changes [22, 23]. Catastrophic thrombosis occurs in patients with cancer; the combination of CAPS and malignancy has been described in several patients. Recurrent thromboembolic events and antibodies against platelet factor 4/heparin complexes have been described, despite no recent exposure to heparin, an entity referred to as spontaneous heparin-induced thrombocytopenia (HIT) [24]. A small subset of patients with thrombotic storm have no recognized associated hypercoagulable states, a condition referred to as "idiopathic" thrombotic storm [21].

The initial diagnostic evaluation of a patient presenting with multiple thrombotic events includes imaging studies to define the location and extent of thrombotic occlusions, which may guide subsequent targeted therapeutic interventions and laboratory studies that include a complete blood count and blood film, screening coagulation studies (prothrombin time, activated partial thromboplastin time [aPTT], and fibrinogen), a metabolic profile, and testing for aPL. For patients in whom the initial assessment raises concern for occult malignancy, additional diagnostic imaging and/or laboratory studies should be obtained to confirm the diagnosis [25].

Therapeutic anticoagulation must be initiated promptly, as new thromboses occur rapidly. Unfractionated heparin is an effective anticoagulant in this setting, given the ability to titrate dose, familiarity with management in the periprocedural setting, and availability of a reversal agent (which should be reserved for cases of disastrous hemorrhagic complications only). A parenteral direct thrombin inhibitor, such as argatroban and bivalirudin, can be used if there has been relatively recent exposure to heparin and delayed HIT is in the differential diagnosis. Low molecular weight heparin is effective in the majority of patients with multiple thromboses and malignancy, assuming normal renal function.

Many patients will have thrombocytopenia on presentation. If review of the peripheral blood film by the hematologist reveals schistocytes, the patient most likely has a

thrombotic microangiopathy, such as TTP or CAPS. A prolonged aPTT may suggest the presence of an aPL, but this needs to be further evaluated with additional laboratory testing. If either diagnosis is suspected, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) level should be sent and plasma exchange should be initiated; a diagnosis of TTP is supported by a markedly decreased ADAMTS13 level (e.g., <5%). Corticosteroids are also commonly administered to patients with CAPS [17] as well as acquired (autoantibody mediated) TTP [26].

Patients with a new or unexpected thrombocytopenia on presentation in the absence of a microangiopathic hemolytic anemia, or who rapidly exhibit a drop in the platelet count after heparin is initiated, may have an unusual presentation of HIT, referred to as spontaneous HIT [24]. Laboratory testing for anti-platelet factor 4/heparin antibodies should be sent, and the patient should be switched to an alternative anticoagulant, either a parenteral direct thrombin inhibitor or fondaparinux.

If the prothrombin time (PT) is also prolonged, and/or the fibrinogen level is decreased, the possibility of a consumptive process, such as DIC, needs to be considered. Depending on the severity of the coagulopathy, DIC can complicate safe administration of anticoagulant therapy [21]. Another cause of an elevated PT is antibody to prothrombin, which sometimes accompanies aPL, whether or not the patient has CAPS. If this antibody is the cause, corticosteroid therapy is indicated.

Management of Catastrophic Antiphospholipid Syndrome

The current recommendations for the treatment of CAPS are based on the following steps:

A. Identify and treat the precipitating factor, such as infection [27] or perioperative discontinuation of anticoagulation [28].
B. Decide if the patient needs supportive measures, such as hemodialysis, mechanical ventilation for respiratory failure, or inotropic drugs [29].
C. Combine anticoagulation (full-dose unfractionated or low molecular weight heparin) with glucocorticoids (GC, intravenous pulses [500–1000 mg/day of methylprednisolone or equivalent for 1–3 days] or oral or intravenous doses of 1–2 mg/kg/day of prednisone or methylprednisolone or equivalent) [30]. If the clinical course is satisfactory, maintain heparin for at least 7–10 days; after which consider substituting with oral anticoagulation (warfarin or other vitamin K antagonists; the use of the direct-acting oral anticoagulants cannot be recommended until there is better information regarding their efficacy and safety in this condition). If the baseline aPTT is prolonged, adequacy of heparin anticoagulation should be confirmed with an anti-factor Xa assay (targeting the upper end of the recommended heparin level of 0.3–0.7 anti-factor Xa units/mL).
D. Consider plasma exchange (PE) and/or intravenous immunoglobulin (IVIG) in cases of life-threatening situations or signs of microangiopathic hemolytic anemia [14, 30, 31]. (We use 5% albumin solution as a choice replacement fluid for PE.) There are no dosing recommendations on the dose of IVIG; suggested

doses range from 400 mg/kg once at the low end up to 400 mg/kg daily for 5 days [total dose of 2 g/kg] at the high end, sometimes continued monthly. Many clinics administer IVIG together with plasma exchange, spacing interventions to allow IVIG to have its effect before removing it [32].

E. Consider intravenous cyclophosphamide (750 mg/m^2 monthly or 500 mg every 2 weeks during 6 or 3 months, respectively) in patients with associated SLE, especially in those with active disease [33].
F. In unusually severe or refractory cases, consider rituximab as an add-on treatment [34]. Eculizumab may be an option if signs of thrombotic microangiopathic hemolytic anemia are present [35].

The 14th International Congress on aPL Task Force on CAPS recommended the triple therapy (anticoagulation + GC + PE and/or IVIG) with a grade B recommendation [1]. In addition, for patients with SLE or another systemic autoimmune disease, quadruple therapy (anticoagulation + GC + PE and/or IVIG + cyclophosphamide) may be beneficial, with a grade D recommendation [36]. Regarding rituximab, the members of the Task Force stated that it has a good safety profile in patients with CAPS and that it may have a role as a second-line therapy in patients refractory to standard triple therapy [1]. These recommendations were based on the analysis of patients with CAPS included in the CAPS Registry. Given the low prevalence of CAPS (around 1% of all cases of APS), it is difficult to analyze this condition in formal prospective studies or to design appropriate clinical trials.

What is the rationale for these treatment recommendations? Glucocorticoids may overcome the excessive cytokine response in these patients. Anticoagulation prevents the thrombosis associated with aPL, PE removes aPL and possibly proinflammatory cytokines, and IVIG reduces aPL titer and the levels of proinflammatory cytokines. Rituximab, blocking CD20, may decrease generation of aPL, and eculizumab, inhibiting the C5 complement protein, prevents C5b-C9 complex generation. Despite these theoretical arguments, only indirect evidence exists about the efficacy of the triple therapy. It seems possible that an inflammatory state promoted by a "cytokine storm" is present in patients with CAPS, given the higher levels of ferritin, an acute phase reactant [15], soluble P-selectin, and von Willebrand factor activity during the catastrophic event compared to the quiescent phase [37]. As a consequence, proinflammatory cytokines may lead a procoagulant effect by inducing tissue factor expression on mononuclear cells and endothelial cells.

Anticoagulant therapy should not be discontinued, even if bleeding occurs (unless life threatening), since discontinuation is associated with rapidly progressive thrombotic events [18, 20] that need to be treated quickly and aggressively. Anticoagulant therapy should be reassessed and adjusted downward if necessary. For extensive or life-threatening thromboembolic events, such as massive pulmonary embolism, fibrinolytic therapy may be useful. Alternative immunomodulatory strategies may be necessary for patients with an autoimmune mechanism contributing to the thrombotic events, including rituximab or intravenous immunoglobulin.

For those who survive the initial events, the majority do well without recurrence. Because of the severity of CAPS, most survivors are advised to remain on chronic anticoagulant therapy.

Group Conclusions and Future Directions

Catastrophic APS is a rare disorder. Its rarity, the lack of knowledge or orientation among treating physicians (mostly, intensive care unit physicians), and the lack of animal model make it difficult to develop an evidence-based model of its pathogenesis. Our knowledge comes from relatively small and retrospective series and case studies. Hopefully, with a more widespread recognition of this condition, more patients will be identified and investigated, elaborating our knowledge on molecular pathways leading to CAPS. So far, the CAPS Registry that compiles all published cases as well as those reported by their physicians in charge is the best way to create information about CAPS [17].

Regarding treatment, points that need to be clarified in CAPS patients are the best steroid dose and tapering schedule, the best replacement fluid during the PE sessions, the best therapeutic dose and moment to administrate IVIG, the role of rituximab and new anticoagulants, and the effectiveness of adding eculizumab to standard triple therapy with or without thrombotic microangiopathy [38]. Finally, an evidence-based medicine, systematic approach is currently being performed with the support of the European Union and the World Health Organization, to elaborate new guidelines for the diagnosis and management of CAPS [39].

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Chapter 18
15th International Congress on Antiphospholipid Antibodies Task Force on Antiphospholipid Syndrome Treatment Trends Report

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Introduction

Antiphospholipid syndrome (APS) is characterized by arterial and/or venous thrombosis, pregnancy morbidity, and persistent antiphospholipid antibodies [1]. Thrombosis and pregnancy morbidity (recurrent embryonic or fetal loss, preeclampsia [PE], and intrauterine growth restriction [IUGR]) compose the criteria manifestations. Non-criteria manifestations (livedo reticularis, thrombocytopenia, hemolytic anemia, cardiac valve disease, nephropathy, skin ulcers, and cognitive dysfunction) are also part of the disease spectrum. Catastrophic antiphospholipid syndrome (CAPS) is a rare but severe disease manifestation associated with high mortality.

Anticoagulation with vitamin K antagonists (VKA) is conventional therapy for secondary thrombosis prevention, but strict laboratory monitoring, dietary modifications, and medication adherence are required for optimal treatment. Despite anticoagulation, thrombosis recurrence can be as high as 40% after 10-year follow-up [2]. Non-criteria manifestations are usually refractory to anticoagulation; their management is based on anecdotal experience.

The goal of the 14th International Congress on Antiphospholipid Antibodies (Rio De Janeiro, Brazil, September 2013) Task Force on APS Treatment Trends was to offer opinions on potential new treatment strategies, other than conventional anticoagulants or antiplatelet agents. The task force members systematically reviewed in vitro, animal, and completed or ongoing clinical studies in aPL-positive patients. Recommendations were presented in open discussions before and during the congress, following which the task force report was finalized and published [3].

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The goal of the 15th International Congress on Antiphospholipid Antibodies (www.apsistanbul2016.org) (North Cyprus, September 2016) Task Force on APS Treatment Trends was to update potential future treatments. The task force members again systematically reviewed the most recent literature, presented recommendations during the Congress, and finalized the report. It is organized in two parts: (a) update on the treatments included in the first report (please refer to the original report for detailed information) [3] and (b) new treatments and/or pathways for consideration.

Part A: Update on Treatments Discussed Previously

Direct Oral Anticoagulants

Direct oral anticoagulants (DOACs) include the direct thrombin inhibitor dabigatran etexilate and the direct anti-factor Xa inhibitors rivaroxaban, apixaban, and edoxaban. These agents, unlike warfarin, are prescribed in fixed doses with more predictable anticoagulant effects, do not interact with diet or alcohol, and have fewer reported drug interactions that affect anticoagulant intensity. Furthermore, monitoring of anticoagulant intensity of a DOAC is not routinely required because anticoagulant effects are more predictable.

The 14th International Congress on aPL Task Force on Treatment Trends concluded that "warfarin or other VKA remains the mainstay of anticoagulation in thrombotic APS. Direct oral antiocagulants can be considered in APS patients with a first or recurrent venous thromboembolism (VTE) occurring off or on subtherapeutic anticoagulation, only when there is known VKA allergy/intolerance or poor anticoagulant control. There were no data to recommend DOACs in APS patients with recurrent VTE occurring on therapeutic anticoagulation or with APS-related arterial thrombosis" [3].

A systematic review (through April 2016) presented during the 15th International Congress on aPL identified seven case reports and four case series. They included 99 DOAC-treated APS patients, of whom 38 had primary APS, 23 APS associated with lupus, and 38 unspecified. Approximately 20% of patients had vascular events during a mean follow-up of 12 months [4]. More recently, in a prospective case series, six (four triple aPL-positive) of 56 (11%) APS patients developed recurrent thrombosis on DOAC (mean follow-up 22 months) [5].

The anecdotal clinical reports (in which rivaroxaban was used in the majority of patients), with recognition of their inherent limitations of publication bias and low evidence level study designs, suggest that recurrent thrombotic events with DOACs in APS patients mainly occur when DOACs are used for secondary prevention of aPL-related arterial thrombosis or microthrombosis, situations where DOACs are not approved. They highlight the need for randomized controlled trials to guide the use of DOACs in thrombotic APS. One recently completed and two ongoing controlled clinical trials and a feasibility study on DOACS were presented during the Congress.

Rivaroxaban in Antiphospholipid Syndrome (RAPS) Trial

The Rivaroxaban in Antiphospholipid Syndrome (RAPS) trial (principal investigator [PI], Hannah Cohen) compared rivaroxaban to warfarin (at a target international normalized ratio [INR] of 2.5, range 2.0–3.0) to treat patients with previous VTE; RAPS was a randomized, controlled, open-label, phase II/III, non-inferiority trial [6]. Eligible patients had taken standard-intensity warfarin for at least 3 months after the last venous thromboembolism (VTE). Exclusion criteria were arterial thrombosis, recurrent VTE while taking warfarin at a therapeutic INR, and age younger than 18 years. Warfarin-treated APS patients with previous VTE, with or without SLE, were randomized 1:1 to warfarin or rivaroxaban, 20 mg once daily, stratified by center and SLE/non-SLE.

The definition of non-inferiority was not VTE recurrence rate but thrombin generation testing, which assesses in vitro the inhibitory effects of anticoagulants. The primary outcome measure was percentage change in endogenous thrombin potential (ETP, the area under the thrombin generation curve) from randomization to day 42, with treatment continued for 180 days and follow-up for 210 days.

One hundred sixteen patients were randomized. Judged by the primary outcome, percentage change in ETP, rivaroxaban was inferior to warfarin. However, because peak thrombin generation was lower with rivaroxaban, the overall thrombogram suggested no difference in thrombotic risk. No new thrombotic events were seen during 6 months of treatment. No patients had major bleeding; clinically relevant and minor bleeding rates were similar in the two groups.

A limitation of the RAPS is that it used a laboratory surrogate outcome measure. The intended selection bias, limiting the selection of patients to those with previous VTE leading to treatment with standard-intensity warfarin, and which ensured a clinically homogeneous study population with definite APS, was a strength. The authors cautioned that the results do not apply to APS patients with venous thrombosis who require higher intensity anticoagulation and to APS patients with arterial thrombosis.

Rivaroxaban in Thrombotic Antiphospholipid Syndrome (TRAPS) Trial

The objective of the ongoing TRAPS trial (PI, Vittorio Pengo) is to demonstrate non-inferiority of rivaroxaban 20 mg (15 mg in patients with moderate renal insufficiency) daily versus warfarin (INR 2.0–3.0) with respect to cumulative incident thrombosis (arterial or venous) confirmed by imaging studies, major bleed, and death in triple aPL-positive APS patients. The trial is multicenter, interventional, prospective, parallel, randomized, controlled, and open-label trial; it plans to recruit 535 patients [7].

Rivaroxaban in Antiphospholipid Syndrome (RAPS) Pilot Feasibility Study

The rivaroxaban in APS pilot feasibility study (PI, Mark Crowther) (clinicaltrials.gov#: NCT02116036) is a prospective cohort study for patients with confirmed APS and prior VTE, with or without prior arterial thrombosis, allocating

them to receive rivaroxaban, 20 mg daily. Patients are followed for thrombosis. The study is designed as a feasibility study with clinical outcomes as secondary endpoints (thrombosis; minor, major, and fatal bleeding). Recruitment was closed on September 30, 2016, with a plan to follow all patients for 1 year. Seventy-nine patients were identified, far below the expected recruitment of 150, suggesting that future studies will have to employ many centers and be international. To date few complications, and no recurrent thromboses, have occurred. One patient suffered unexplained hepatitis. This Canadian RAPS study will be underpowered but will add to the body of evidence on safety and efficacy of DOACs in patients with APS.

Apixaban for the Secondary Thrombosis Prevention in Antiphospholipid Syndrome (ASTRO-APS)

ASTRO-APS (PI, Scott Woller) is a prospective, randomized, open-label, blinded pilot study comparing apixaban with dose-adjusted warfarin (target INR range 2.0–3.0) for the secondary prevention of thromboembolism among patients with a history of APS and thrombosis. The intentions of this phase IV pilot study are to provide data on feasibility of enrolling APS patients and to estimate efficacy and safety of apixaban compared with usual care [8].

ASTRO-APS was originally designed to compare apixaban 2.5 mg twice a day with dose-adjusted warfarin, enrolling patients with history of arterial or venous thromboses receiving indefinite anticoagulation. The primary clinical outcomes are rates at 1 year of arterial or venous thrombosis, death caused by thrombosis, major bleeding, and clinically relevant nonmajor bleeding.

After accrual of the first 25 patients, a prespecified Data Safety Monitoring Board (DSMB) review recommended the protocol be modified to use apixaban 5 mg twice a day. After five more patients were enrolled, a potential safety signal led to an ad hoc DSMB rereview, which recommended: first, to continue ASTRO-APS; second, exclude patients with prior arterial thrombosis; and, third, obtain brain magnetic resonance imaging with stroke protocol for all otherwise eligible candidates to exclude prior silent stroke. ASTRO-APS plans to enroll 200 patients.

15th International Congress on Antiphospholipid Antibodies Task Force on Treatment Trends Recommendation

Insufficient evidence exists to make recommendations at this time regarding DOAC use in APS. The RAPS trial suggests that rivaroxaban might be useful in selected APS patients with single venous thromboembolism requiring standard-intensity anticoagulation; however, this needs to be confirmed with additional studies using clinical outcome measures.

Statins

Benefit of statins in primary and secondary prevention of coronary heart disease is proven, which is due to the lipid-lowering effect of these drugs and to their immunomodulatory, anti-inflammatory, and antithrombotic properties [9]. Statins have multiple effects on monocyte, lymphocyte, and endothelial cell activities that may contribute to thrombosis prevention in APS. Antiphospholipid antibodies induce expression of tissue factor (TF) and cell adhesion molecules; fluvastatin, simvastatin, and rosuvastatin reduce this expression [10]. In a mouse model of obstetrical APS, simvastatin and pravastatin reduced fetal death by inhibiting TF and protease-activated receptor 2 (PAR2) expression on neutrophils [11]. In a thrombosis mouse model, fluvastatin reduced thrombus size [12].

Based on in vitro and two human studies using surrogate markers [13, 14], the 14th International Congress on aPL Task Force on Treatment Trends concluded that "although statins ameliorate the proinflammatory profile and down-regulate the prothrombotic stage found in APS patients, based on the available data, statins cannot be recommended in APS patients in the absence of hyperlipidemia. However, a subgroup of aPL-positive patients with recurrent thrombosis despite adequate anticoagulation might derive benefits from statins [3]."

Since then there have been no systematic studies with statins in thrombotic APS. A study presented at the 15th International Congress on aPL [15] reported 21 pregnant APS patients who developed preeclampsia (PE) and/or IUGR on low-dose aspirin (LDA) and low molecular weight heparin (LMWH). Of those studied, 11 received pravastatin (20 mg daily) initiated at the onset of PE and/or IUGR (in addition to LDA + LMWH) and 10 did not. The study was not randomized, and not all patients met APS criteria at the time of enrollment. All pravastatin-treated patients had live births near full term. In the 11 patients who did not receive pravastatin, 11 deliveries were preterm, and five neonates died, and three of the six survivors had abnormal development. Patients treated with pravastatin had increased placental blood flow and improvements in PE features as early as 10 days after treatment was begun.

The rapid improvement in uterine artery hemodynamic parameters in pravastatin-treated patients (uteroplacental perfusion was assessed by Doppler) suggests that the drug targets placental vasculopathy, possibly by stimulating release of vasoactive substances, such as nitric oxide, from the endothelium [16]. Statins also downregulate TF, the major initiator of the coagulation cascade in vivo and a crucial molecule linking inflammation and thrombosis in APS [17]. These protective effects may explain the amelioration of placental and maternal PE [18].

15th International Congress on Antiphospholipid Antibodies Task Force on Treatment Trends Recommendation

No controlled clinical trial supports the use of statins in APS patients. Because statins downregulate prothrombotic and proinflammatory biomarkers, statins may be used in APS with high risk for cardiovascular events and in those with recurrent thrombosis despite adequate anticoagulation.

Statins are classified as Category X (contraindicated) for pregnancy by the US Food and Drug Administration, because of the disruption of gonadal stem cell development and theoretical long-term fetal neurological damage [19]; however, several recent studies did not find teratogenic effects [20, 21]. An American national study on drugs in pregnancy [21] did not find an increase in congenital malformations or organ-specific malformations among the offspring of the 1152 women exposed to statins during the first trimester; the relative risk of malformation was 1.79 (95% confidence interval 1.43–2.23), which fell to 1.07 when controlled for the confounders such as diabetes. The task force does not suggest the use of statins during APS pregnancies without further efficacy and safety data.

B-Cell Inhibition

Animal models demonstrate that B cells play an important role in the pathogenesis of APS. Blocking B-cell-activating factor (BAFF) prevents disease onset and prolongs survival in APS murine models [23]. Rituximab and belimumab are the only B-cell-inhibiting biologic therapies whose effect has been studied in APS patients.

Based on the case reports [23–25], CAPS registry data [26], and one open-label uncontrolled pilot study [27], the 14th International Congress on aPL Task Force on Treatment Trends concluded that "B-cell inhibition may have a role in difficult-to-treat APS patients, possibly in those with hematologic and microthrombotic/microangiopathic manifestations [3]."

Since then, although there have been case reports/series of rituximab use in APS and a recent report of belimumab use in two primary APS patients [28], there is not yet a prospective study of B-cell inhibition in APS.

15th International Congress on Antiphospholipid Antibodies Task Force on Treatment Trends Recommendation

No change in recommendations.

Complement Inhibition

Complement activation contributes to thrombosis in APS animal models. Antiphospholipid antibodies activate complement factor 5 (C5), generating fragment 5a (C5a), which induces adhesion molecules and TF in inflammatory cells and platelets and triggers release of prothrombotic and pro-inflammatory mediators [29, 30]. In animal models of APS, C5a interacts with its receptor (C5aR), amplifying endothelial cell activation and vascular inflammation, promoting trophoblast injury and angiogenic factor imbalance, and producing microthrombotic lesions [29, 31,

32]. Inhibition by anti-C5 antibody, C5aR antagonist peptides, and anti-C5aR antibody protects mice from pregnancy losses and from thrombosis [33]. Mice treated with aPL but deficient in *complement regulatory components*, i.e., mice in which the complement cascade is not normally inhibited, have poor pregnancy outcomes, including thrombotic microangiopathy. Mice treated with aPL, but deficient in *complement components*, have good pregnancy outcomes. These contrasting experiments indicate that complement is an important mediator in pathogenesis of both manifestations of APS [34]. Heparin, used in APS patients to treat thrombosis and prevent miscarriages, has a complement inhibitory effect [35].

Given the reports that complement inhibition improves outcomes in mouse models and CAPS patients [36], the 14th International Congress on aPL Task Force on Treatment Trends concluded that "complement inhibition may have a role as an adjuvant or main therapy for APS patients refractory to anticoagulation; however more clinical data are needed before this medication can be recommended" [3].

Since then a phase IIa study of treatment of non-criteria manifestations of APS (nephropathy, thrombocytopenia, and skin ulcers), designed to evaluate safety of an intravenous C5a inhibitor (clinicaltrials.gov #: NCT02128269), has ended, but the results are not yet available. Another phase II study using eculizumab (a C5 inhibitor) to prevent thrombosis after renal transplantation in patients with prior CAPS (clinicaltrials.gov#: NCT1029587) is currently recruiting.

Eculizumab is a fully recombinant humanized hybrid IgG2/IgG4 monoclonal antibody that binds to C5 and inhibits C5 cleavage by C5 convertase, thereby inhibiting the inflammatory, thrombotic, and lytic functions of complement. Eculizumab is FDA-approved for paroxysmal nocturnal hemoglobinuria (PNH), an illness in which red blood cells and leukocytes are prone to complement-mediated lysis due to deficiency in CD55 or CD59, and for atypical hemolytic-uremic syndrome (HUS) [37]. A case report described complement inhibition in a refractory patient with CAPS who underwent kidney transplantation [38], and several other cases have been published [36]. Kidney allograft survival improves if complement blockade is initiated at the time of transplantation [39], suggesting a use in patients with APS nephropathy undergoing renal transplantation.

Complement plays a role in ischemia/reperfusion injury, antibody- and cell-mediated transplant rejection, C3 glomerulopathy, and atypical HUS [40]. Eculizumab was used to block thrombotic microangiopathy (TMA) in a patient with atypical HUS [41]. Three patients with APS (two with CAPS) treated with anticoagulation and eculizumab prior to and after renal transplantation had functioning renal allografts, with no systemic thrombotic events or early graft losses [42], confirmed in a report demonstrating improvement in TMA after eculizumab in APS nephropathy that recurred after kidney transplantation. In this case pretreatment intense C5b-9 and C4d deposit in kidney biopsies were absent after treatment with eculizumab, but chronic vascular renal changes were not prevented [43]. A recent case report described the potential use of eculizumab to prevent re-thrombosis in an arterial bypass graft in APS. Immunofluorescence showing β_2-glycoprotein-I (β_2GPI) on the endothelium of the artery wall suggested a pathogenic role for aPL [44].

Rivaroxaban, a direct factor Xa inhibitor, decreases markers of complement activation (C3a, C5a, and soluble [s] C5b-9) [45]. Antiphospholipid syndrome patients had higher baseline C3a, C5a, and SC5b-9 and, after 42 days of rivaroxaban treatment, a statistically significant decrease was observed (compared to controls treated with warfarin), possibly because rivaroxaban inhibits FXa-induced complement activation of C5 [46]. Further studies are necessary to confirm these findings.

Recently a novel recombinant antibody recognizing the first domain of β_2GPI was shown to induce thrombosis and fetal loss in animal models [47]; a non-complement fixing CH2-deleted variant of that antibody reduced the detrimental effects, suggesting another complement-mediated mechanism and another possible treatment for refractory APS [47].

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Although there are encouraging reports about efficacy of complement inhibition in APS, publications may be biased toward positive results. At this time, patients with life-threatening disease resistant to other interventions may be candidates for complement inhibitors as salvage therapy.

Peptide Therapy

All proposed peptide therapies are based on the premise that the key pathogenic interaction in patients with APS occurs when aPL engages one of the five domains (DI–DV) of β_2GPI [48]. Antibodies to several domains occur in APS patients, but most pathogenic aPL bind the N-terminal DI [49]. Each antigen-binding arm of the antibody can bind a separate β_2GPI molecule, creating a dimeric structure that binds anionic PL on cell membranes, thus interacting with cell surface receptors like Toll-like receptors (TLR) and apolipoprotein E receptor 2 (ApoER2) to alter cell function [50]. In the absence of aPL, β_2GPI is monomeric, is present constitutively in human serum, and does not exert these effects. The peptide agents proposed as new therapies for APS all act by blocking binding at different points. It is unknown which approach is best.

The 14th International Congress on aPL Task Force on Treatment Trends task force recommended that “at present, peptide therapy is not ready for trials in patients; however peptide therapy is potentially an important future targeted treatment for aPL-positive patients. Chemical modification to improve half-life and minimize immunogenicity will be required. Different peptides may be needed for different aPL manifestations” [3].

Since then, no peptide therapies have yet entered human trials. Considerable information from in vitro and animal studies suggests that peptides may become therapeutic agents in the future.

It is unknown which domain of β_2GPI may be the best therapeutic target. The key epitope for aPL on DI lies between the arginine residues R39 and R43 [49, 51]. In the in vivo femoral vein thrombosis model developed by Silvia Pierangeli, some DI peptide analogues inhibited thrombosis, TF expression in peritoneal macrophages, and aortic VCAM-1 expression, whereas others did not [52].

Linear DI peptides containing the critical R39–R43 epitope do not bind APS-IgG as well as does whole DI [53, 54]. McDonnell et al. have published a method for producing recombinant DI in bacteria [55] and have improved its half-life by adding polyethylene glycol group PEGylation [56, 57].

An alternative idea is to block DV binding to phospholipids. The octapeptide CKNKEKKC inhibits binding aPL to cardiolipin [58]. A 15-mer peptide from DV (called GDKV) and a cytomegalovirus peptide (TIFI) homologous to GDKV have been used as inhibitors. In mouse models that use injected aPL, TIFI inhibits thrombosis in the femoral vein [59] and reduces fetal loss [60]. TIFI also reduces binding of β_2GPI to human umbilical vein endothelial cells (HUVEC) [59] and to human trophoblast cells [60].

Blank et al. joined two synthetic peptides derived from DV (one was GDKV) with a flexible linker molecule [61]. When HUVEC were preincubated for a short while with the construct, binding of β_2GPI/anti-β_2GPI antibody (aβ_2GPI) complexes to HUVEC was reduced by 89%, but the reduction in binding was lost with prolonged preincubation because the construct was taken up in the cells, thus tempering enthusiasm for use of this agent.

Kolyada et al. developed a dimer, the A1 ligand-binding module of the ApoER2 receptor, that binds DV of β_2GPI [62–64]. Their construct blocks binding of both β_2GPI/ aβ_2GPI and DV alone to cardiolipin far more strongly than does monomeric A1 but does not do so in the absence of aβ_2GPI; it reduces thrombosis induced by laser trauma in two mouse models [63]. Recently the same group developed a mutant dimer that inhibits binding and clotting more strongly than does the wild type [64].

Recombinant DI, TIFI, and A1-A1 are all credible peptide therapies for APS. Proponents of TIFI and A1-A1 could argue that the heterogeneity of the aβ_2-glycoprotein-I in patients with APS mitigates against an approach that targets DI alone. Conversely, others might argue that interfering with the interaction of DV with PL may have adverse effects on physiological processes that occur in the absence of aPL. Perhaps there is a need for different peptide therapies. There is little information about potential toxic effects of any of these agents. All putative peptide therapies will have to be modified to enhance half-life [63].

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The key research needs are to take one or more of these agents forward to formal pharmacokinetic and toxicology studies, then to a first-in-man trial.

Vitamin D

A growing body of evidence highlights vitamin D's immunomodulatory properties. Vitamin D insufficiency (<30 ng/ml) occurs in up to 70% of patients with APS and/or SLE, and vitamin D deficiency (<10 ng/ml) occurs in 11–50%. Low vitamin D levels in APS patients correlate with venous and arterial thrombosis and with non-criteria manifestations. Values in thrombotic APS patients are lower than in obstetric APS patients [65–67]. However, low-dose short-term vitamin D supplementation in a small group of primary APS patients was ineffective in raising levels above 30 ng/ml [67]. Human umbilical vein endothelial cell cultures demonstrate that vitamin D inhibits expression of prothrombotic TF in response to aβ_2GPI stimulation [65], a possible mechanism for therapeutic effect.

The 14th International Congress on aPL Task Force on Treatment Trends task force recommended that "vitamin D deficiency and insufficiency should be corrected in all aPL-positive patients based on the general population guidelines. The prognostic role of vitamin D deficiency and therapeutic value of supplementation (including the dosage and definition of treatment goals) in aPL-positive patients should be clarified with prospective studies that include appropriate control groups and standardized definitions of vitamin D deficiency" [3].

There have been no ongoing studies evaluating the effect of vitamin D in aPL-positive patients. However, a randomized clinical trial of vitamin D prophylaxis in the prevention of hypertensive disorders of pregnancy (clinicaltrials.gov #: NCT02920593) recently began. Investigators will also evaluate placental pathology and measure placental levels of several proinflammatory markers.

In the early stages of pregnancy, trophoblasts respond to and produce vitamin D, promoting an anti-inflammatory environment and inducing decidualization [68–70]. Vitamin D deficiency is associated with an increased risk for preeclampsia and IUGR [71]. A retrospective cross-sectional study of women with recurrent pregnancy loss (RPL) stated that vitamin D deficiency and insufficiency are associated with aPL, elevated peripheral blood natural killer (NK) cells, and elevated NK cell cytotoxicity [72]. In vitro studies confirmed the ameliorative effect of vitamin D on NK cell cytotoxicity and Th2-type response, both associated with successful pregnancy outcomes [72, 73].

Studies utilizing a human first trimester trophoblast cell line and primary trophoblast cultures show that vitamin D treatment alone or combined with LMWH limits aPL-mediated inflammatory response [74]. Vitamin D also reduces the elevated anti-angiogenic factor sFlt-1 seen in aPL- and/or LMWH-treated trophoblasts and placental villi explants [74–76]. The importance of this finding lies in the strong association of sFlt-1 with preeclampsia. Elevated levels of sFlt-1 are also seen in pregnant women treated with LMWH [77–78], possibly explaining the contradictory results of studies on the effectiveness of LMWH and aspirin in aPL-associated pregnancies [79] and suggesting a role for adjunctive vitamin D in LMWH-treated obstetric APS.

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No change in recommendations.

Part B: New Treatments/Pathways for Consideration

Mechanistic Target of Rapamycin (mTOR) Pathway Inhibitors

Lesions affecting the renal microarchitecture (thrombotic microangiopathy or chronic vaso-occlusive lesions), known as aPL-associated nephropathy (APSN), represent a non-criteria APS manifestation [80, 81]. The mTOR pathway is a potential intermediate in its development [82].

Activation of mTOR in endothelial cells is associated with proliferation of endothelial and vascular smooth muscle cells. Sirolimus prevents mTOR activation and, when used in APS transplant recipients, is associated with reduction of vascular cell proliferation and APSN renal lesions and preserved renal function [82].

Recruitment of the Akt/mTOR pathway in endothelial cells is triggered not only by APS but also occurs in conditions associated with injuries to the endothelium, such as mechanical trauma or anti-HLA antibodies after organ transplantation [83], suggesting that this is a common final pathway for endothelial injury and thus a potential therapeutic target [84, 85], an assertion supported by retrospective cohort analyses but in need of prospective study confirmation.

Evidence regarding the impact of mTOR inhibition in APSN is based on allografts. In non-transplanted patients with APSN who have impaired renal function and proteinuria, mTOR inhibitors may worsen proteinuria and glomerular lesions. In fact, evidence suggests that sirolimus-induced proteinuria is linked to inhibition of mTOR2-dependent Akt2 phosphorylation in podocytes; its detection might predict occurrence of this side effect [86].

In allografted APS patients, APSN recurrence may occur early, suggesting that ischemia/reperfusion injury affects endothelial cells, with recruitment of complement pathway leading to TMA and/or phospholipid exposure, thus leading to cell activation via the Akt/mTOR pathway. That sirolimus prevents development of vascular lesions [82] suggests that a sirolimus-based regimen is preferable to one based on calcineurin inhibitors. In vitro studies also suggest a link between aPL and mTOR pathway activation [82], possibly through the stretching of the cell membrane [87]. In the cited in vitro study, all patients had lupus anticoagulant, anticardiolipin antibodies (aCL), and aβ_2GPI and titers of the antibodies correlated with the degree of mTOR pathway activation, specifically via AKT phosphorylation on residue Ser^{473}. Although eculizumab may favorably impact posttransplant APS-related TMA, it does not prevent development of other APS vascular lesions [43].

Integrin Inhibitors

Integrins are heterodimeric membrane receptors composed of an alpha- and a beta-subunit. The platelet-specific integrin αIIbβ3 plays a critical role in platelet activation and is centrally involved in hemostasis and thrombosis. Alpha-IIbβ3 changes its conformation in response to prothrombotic stimuli, switching from a low- to a high-affinity receptor for its ligands, including fibrinogen and von Willebrand factor (VWF). Fibrinogen and VWF bind to αIIbβ3, mediating platelet aggregation, while its inhibition prevents thrombus generation [88, 89].

Three intravenous αIIbβ3 inhibitors are available: abciximab, eptifibatide, and tirofiban; orally administered αIIbβ3 inhibitors are ineffective.

(a) *Abciximab:*

Abciximab is a glycoprotein IIb/IIIa receptor antagonist monoclonal antibody approved to prevent thrombosis that may occur during percutaneous coronary intervention (PCI). Although abciximab decreases the risk of periprocedural complications, its efficacy in improving long-term outcomes is controversial [89–91]. This treatment may reduce the risk of restenosis in diabetic patients undergoing PCI [92], possibly because it has anti-inflammatory properties [89].

Data regarding abciximab use in APS are scant. Case reports describe a young aPL-positive woman with a large carotid bifurcation embolus causing >90% stenosis who was successfully treated with urokinase followed by abciximab [93] and another patient with acute myocardial infarction treated with thrombectomy and abciximab [94]. There are no clinical trials assessing the effectiveness of this drug in patients with aPL.

Endovascular procedures, leading to endothelial disruption and activation, can act as triggers for clot formation in aPL-positive subjects [95]. Use of abciximab can be considered in this context, but further studies are needed to define its potential indication.

(b) *Eptifibatide:*

Eptifibatide is a cyclic heptapeptide able to inhibit fibrinogen binding to αIIbβ3 [88, 89, 96]. Its plasma half-life is between 1.5 and 2.5 h; it is mainly excreted by the kidney. Eptifibatide prevents platelet aggregation, reaching its peak of effect about 15 min after injection. It reduces mortality in patients with acute coronary syndrome (ACS) [89]. Preliminary data suggest that in combination with tissue-type plasminogen activator (tPA), it may be effective for acute ischemic stroke [97, 98]. Intravenous eptifibatide prevents thrombus formation in a laser-induced thrombosis mouse model of APS [99]. There are no data in animals or APS patients.

(c) *Tirofiban:*

Tirofiban is a low molecular weight non-peptide αIIbβ3 inhibitor with a very short biological half-life [89]. It is approved for ACS treatment and has been anecdotally used for acute ischemic stroke [89]. There are no data in animals or APS patients.

Adp P2y$_{12}$ Receptor Antagonists

The binding of ADP with its $P2Y_{12}$ platelet receptor represents a key mechanism of platelet activation, resulting in the reduction of intracellular cyclic AMP (cAMP) concentration that leads to the αIIbβ3 receptor activation [100]. The first commercially available $P2Y_{12}$ ADP receptor inhibitor was ticlopidine, rapidly replaced by clopidogrel because of the latter's more favorable safety profile [101]. Two additional P2Y12 ADP receptor inhibitors, prasugrel and ticagrelor, have, in combination with LDA, recently been approved for patients with ACS and those undergoing coronary stenting [102].

The active metabolites of the prodrugs clopidogrel and prasugrel irreversibly bind to $P2Y_{12}$ ADP receptor, preventing ADP-mediated platelet activation. Several case reports suggest that addition of clopidogrel to warfarin, LDA, or hydroxychloroquine may be beneficial in APS thrombosis patients not responding or intolerant to conventional treatment [102–104]. A Japanese study on 82 APS patients with prior arterial thrombosis suggests that dual antiplatelet therapy with various combinations of clopidogrel, ticlopidine, LDA, and cilostazol reduces the risk of recurrence [105].

Defibrotide

Defibrotide is a mixture of single (90%)- and double (10%)-stranded phosphodiester oligonucleotides derived from depolymerization of porcine intestinal mucosal DNA. The pleiotropic protective effects of defibrotide on endothelial cells (ECs) include profibrinolytic, antithrombotic, anti-inflammatory, and antiapoptotic properties [106]. In in vitro studies in human endothelial cells, defibrotide enhances plasmin activity in hydrolyzing fibrin clots, downregulates P-selectin and monocyte TF expression, and increases tissue plasminogen activator (t-PA) and thrombomodulin (TM) expression and platelet activation modulation [106, 107]. It also interferes with prostaglandin release, increasing synthesis of prostaglandins GI2 and GE2, potent vasodilators, and platelet aggregation inhibitors [107]. Defibrotide is approved for severe veno-occlusive disease following hematopoietic stem cell transplantation [108]. Defibrotide, in combination with other therapies, was used successfully in one CAPS patient [109], published more than 15 years ago.

Cilostazol

Cilostazol is a selective inhibitor of phosphodiesterase-3 (PDE3) approved for treatment of peripheral arterial disease [110]. The increase of cAMP levels and the activation of the regulatory protein kinase A (PKA) resulting from PDE3 blockade

inhibit both primary and secondary platelet aggregation induced by collagen, ADP, and arachidonic acid [111]. Several studies suggest that cilostazol reduces P-selectin expression and induces endothelial nitric oxide synthase (eNOS) [112]. Treatment with cilostazol for 1 year increases levels of the soluble receptor for advanced glycation end products (sRAGE) and decreases concentration of E-selectin, high-sensitivity C-reactive protein (hSCRP), and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with diabetes [113], presumably protecting patients from progressive vascular disease. Cilostazol is used in Japan as a combination therapy; preliminary data suggest that cilostazol has several potentially beneficial therapeutic effects, particularly in patients with arterial involvement. There are no clinical trials assessing its effectiveness in APS patients.

Protease-Activator Receptor (Par) Antagonists

Protease-activator receptor (PAR) antagonists are a family of G protein-coupled receptors that stimulate cell activation in response to serine proteases [114]. Protease-activator receptor-1, PAR3, and PAR4 are recognized by thrombin, which binds to the receptors and irreversibly cleaves them, triggering signal transduction [114]. Protease-activator receptor-1 and PAR4 are expressed by human platelets and play a pivotal role in platelet activation and clot formation. Vorapaxar, a PAR1 inhibitor, is approved as an adjunctive antiplatelet therapy for the treatment of acute coronary syndromes [115–117]. Its use in clinical practice is limited by a high risk of severe bleeding [116]. Another PAR1 inhibitor, atopaxar, in phase II trials, has a smaller risk of bleeding [115].

Toll-Like Receptor (TLR) Inhibitors

Several cell membrane proteins, including Toll-like receptors (TLR) 2 and 4, ApoER2, and annexin A2, are potential mediators of interaction of β_2GPI with ECs [116]. Inhibition of TLR4-mediated cellular signal transduction is a potential treatment for APS. In vitro, TLR4 silencing or knockout in HUVEC reduces aβ_2GPI binding and downregulates E-selectin and ICAM1 expression [117]. In another study, treatment of human blood monocytes with an aβ_2GPI/β_2GPI complex increases TF expression, an effect that was inhibited by a blocker of signaling transduction mediated by the intracellular domain of TLR4 [118].

Similarly, the inhibition of aβ_2GPI interaction with ApoER2 might be postulated in the treatment of APS. Taking into account that β_2GPI interacts with A1, the first ligand-binding domain of ApoER2, the above-cited experiments of Kolyada et al. on peptide inhibitors of β_2GPI binding antibody are relevant [62].

Intracellular Mediator Blockers

Antiphospholipid antibody-mediated intracellular signaling transduction involves phosphorylation of p38 mitogen-activated protein (MAP) kinase and activation of nuclear factor κB (NFκB). Preliminary studies demonstrate that NFκB and/or p38 MAP kinase blockers are promising candidates for treatment of APS. In vitro studies show that p38 MAP kinase and NFκB blockers abrogate aPL-induced TF expression in monocytes, platelets, and HUVEC cultures in vivo [119, 120]; one blocker reduces white blood cells adhesion to EC, TF expression, and thrombus size [121], and another NFκB inhibitor prevents thrombus formation in mouse models of APS [122], suggesting promising treatment options.

Tissue Factor Expression Inhibition

Because aPL-induced upregulation of TF on EC and monocytes may play a role in clot formation [116, 123], inhibition of TF expression may represent a treatment strategy. Dilazep and dipyridamole, two antiplatelet agents, block aPL-induced monocyte TF upregulation [124].

Angiotensin-converting enzyme (ACE) inhibitors reduce TF expression on monocytes in patients with myocardial infarction; recent studies suggest that angiotensin receptor blockers (ARB) attenuate LPS-induced TLR4-mediated inflammation [125, 126].

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Mechanistic Target of Rapamycin inhibitors have a potential to play a role in the management of aPL-positive patients, especially in those with microthrombosis; future mechanistic and clinical studies will identify which aPL-positive patients are likely to benefit from mTOR inhibitors. Despite the promising outcomes reported in case reports, the effectiveness of clopidogrel or other ADP $P2Y_{12}$ receptor antagonists has not been assessed in large cohorts of APS patients; clopidogrel can be considered as an adjunctive therapy in selected APS patients with arterial thrombosis refractory to conventional treatment. There are no clinical studies that assess the effectiveness of integrin inhibitors, defibrotide, cilostazol, protease-activator receptor (Par) antagonists, TLR inhibitors, or tissue factor inhibitors in APS.

Group Conclusions

The APS field is dynamic and moving forward in parallel to better understanding of disease mechanisms. New direct anticoagulants, statins, and B-cell inhibition are potential promising therapies for APS patients, but evidence from large prospective studies is needed. Patients with (non-APS) complement-mediated microthrombotic diseases have benefited from anticomplement therapy, and patients who failed kidney transplantation have improved outcomes with use of mTOR inhibitors. Other important targets lack validation in humans; support for their use is limited to animal or in vitro data.

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Part VI
Antiphospholipid Syndrome for Patients

Chapter 19
Antiphospholipid Syndrome: What Should Patients Know?

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Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder in which the patient's immune system makes antibodies (antiphospholipid antibodies [aPL]) that increase the risk to form blood clots and pregnancy problems. Individuals with antiphospholipid antibodies may or may not develop clinical problems; a positive aPL test alone is not enough to diagnose a patient as having APS. The most common clinical problems are blood clots in the veins of the legs, strokes, and miscarriages. Antiphospholipid antibodies occur in otherwise healthy individuals, in patients who have had certain recent infections, or in patients with other autoimmune disorders such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Primary APS describes those patients with persistently positive aPL and clotting and/or pregnancy events who do not have evidence of another autoimmune disorder or an infection to explain the antibody.

The purpose of this chapter is to provide a general review of APS for patients and those who are interested in learning more about aPL/APS. Detailed information about aPL-related pregnancy problems can be found in Chap. 20.

How Common Are Antiphospholipid Antibodies and Antiphospholipid Syndrome?

In the general population with no systemic autoimmune diseases, up to 10% of individuals may have a positive test, low titer, and transient, which is called clinically nonsignificant aPL (further discussed below); however, clinically significant

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aPL (high titer and persistent) is uncommon (1–2%). In patients with systemic autoimmune diseases, clinically significant aPL are more frequent, in that 30–40% of SLE patients have aPL. In selected parts of the world where mycobacterial and parasitic infections are common, low-titer and transient aPL can occur frequently during infections. Although usually low titer and transient, high-titer clinically relevant aPL can rarely develop.

In aPL-positive individuals with no other systemic autoimmune diseases and no other blood clot risk factors, the yearly risk of clotting is probably very low (<1% per year). However, aPL-positive patients with other systemic autoimmune diseases, such as SLE, have a higher early risk of clotting, which is, however, still low, <4% per year.

About 50% of APS patients have primary APS; they form blood clots and/or have pregnancy problems but are less likely to develop another autoimmune disease. Antiphospholipid syndrome patients with other systemic autoimmune diseases (secondary APS) are usually women; the female predominance is less obvious in primary APS patients.

Antiphospholipid antibodies can be detected in approximately 15% of all stroke patients in the general population, 10% of heart attacks (myocardial infarction) and blood clots in large veins, and 9% of recurrent miscarriages.

What Is the Origin of Antiphospholipid Antibodies?

Usually antibodies are made by the body to fight off bacteria, viruses, or other "foreign invaders" that cause infection. In systemic autoimmune diseases such as APS, the body makes abnormal autoantibodies for reasons we do not yet fully understand but hypothesize that environmental factors, such as infections, trigger these antibodies. Antiphospholipid antibodies are made by the patient's immune system to target the patient's own blood vessels or clotting system proteins (not to target an outside infection); therefore, aPL are known as "autoantibodies" (further discussed below).

Phospholipids are a type of fat molecule that is a normal component of all cells in the body. In APS, a patient's immune system is triggered to make aPL against certain proteins that attach to the phospholipid membrane (cell wall) of cells located on the inner layer of the arteries or veins.

The exact mechanism which prompts the immune system to make aPL is unknown. Systemic infection may play a role in the development of these autoantibodies. One theory suggests that during a bacterial or viral infection, the body's immune system makes antibodies targeted against the bacterial cell membranes, which contain phospholipids. Most of these antibodies are short lived and do not cause clots. However, long after the inciting infection is cleared, some aPL can linger in the blood stream and potentially cause clinical manifestations of APS. For unclear reasons, exposure to certain drugs or some of the cancer cells can also cause the body to make aPL; however, these antibodies are often only transient and found in low levels and rarely cause blood clots.

What Is the Mechanism of Antiphospholipid Antibody-Related Clinical Problems?

One of the main causes of clinical problems in patients with APS is blood clots, usually occurring due to inflammation (a localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury). The mechanism of abnormal clotting is the subject of active research and likely involves multiple mechanisms. Some of the potential mechanisms that are not mutually exclusive include interaction between (a) aPL and the lining of blood vessels resulting in the activation of the cells forming the inner layer of the artery or vein wall, (b) aPL and other molecules involved in the body's natural clotting system causing an imbalance favoring clot formation, and (c) aPL and platelets, which are normally involved in the clotting process. The body has a complicated system involving many different components that work together to prevent both clots and bleeding. Theoretically, aPL interfere with this system at various points thereby shifting the balance toward clot formation. The formation of tiny clots in the placenta is one of the potential mechanisms leading to the various forms of pregnancy morbidity in APS, although the antibodies themselves damaging embryonic cells directly are another important mechanism of aPL-related pregnancy problems.

Given that many people can have aPL in their blood without blood clots being formed, aPL are necessary but likely not sufficient to cause clots without a trigger. Research suggests that the trigger often occurs in the form of damage or stress to a blood vessel. These triggers that can damage blood vessels and make clotting more likely in aPL-positive patients are listed in Table 19.1.

What Are the Clinical Manifestations of Antiphospholipid Antibodies?

There is a wide spectrum of clinical manifestations related to aPL (Table 19.2), and the antibodies can affect any organ system in the body. It is important to note that aPL may occur without any clinical problems, which is commonly referred as "asymptomatic aPL positivity."

Table 19.1 Factors that can increase the risk of blood clots in antiphospholipid antibody-positive patients

Oral contraceptive use, estrogen use, pregnancy
Prolonged immobility, surgical procedures
Older age (>65)
Genetic clotting disorders (e.g., factor V Leiden mutation)
Traditional cardiovascular risk factors (e.g., hypertension, diabetes mellitus, dyslipidemia, smoking, obesity, sedentary lifestyle, early menopause)

Table 19.2 Spectrum of antiphospholipid antibody (aPL)-related clinical problems

I. Asymptomatic aPL-positive individuals[a]
(a) Anticardiolipin antibody IgG or IgM positive in moderate-to-high levels on two or more occasions at least 12 weeks apart
(b) Anti-ß$_2$-glycoprotein-I antibody IgG or IgM positive in moderate-to-high levels on two or more occasions at least 12 weeks apart
(c) Lupus anticoagulant test positive on two or more occasions at least 12 weeks apart
II. Antiphospholipid syndrome (I + below)
(a) One or more episodes of a blood clot in a vein/artery
(b) Pregnancy morbidity
Unexplained fetal loss at or after tenth week of gestation
Premature birth before 34 weeks of gestations because of preeclampsia, eclampsia, or placental insufficiency
Three or more unexplained consecutive miscarriages before 10 weeks of gestation
III. Non-criteria manifestations of aPL (I + below)
(a) Livedo reticularis
(b) Heart valve disease
(c) Low platelet or red blood cell counts
(d) Kidney disease
IV. Catastrophic APS (I + below)
1. Blood clots in three or more organs
2. Development of clots simultaneously or in less than 1 week
3. Evidence of blood clots in small vessel of at least one organ system

[a]Excluding those with chronic infections such as mycobacterium and syphilis

Asymptomatic Antiphospholipid Antibody Positivity

Not all people with positive aPL tests have APS. Often people can have these antibodies and not know it, never develop problems, and feel perfectly well. In such cases, the antibodies were usually incidentally discovered on testing done for other reasons by their doctors. Patients with SLE and other systemic autoimmune diseases are often routinely tested for aPL. In addition, as aPL may cause abnormal activated partial thromboplastin time (aPTT), which is a clotting test used to determine the bleeding risk before surgical procedures, some patients may be found to have aPL during the preparation period for surgical procedures. Also aPL sometimes cause *false*-positive test for syphilis. Since tests for syphilis are often performed in pregnancy and other circumstances, the false-positive test may be the first clue that the antibody is present.

Once aPL are detected, it is important to monitor any new symptoms and eliminate other risk factors for blood clotting as much as possible (further discussed in the management section below).

Antiphospholipid Syndrome

Deep vein thrombosis (DVT) is the most common type of blood clot that forms in veins, and stroke is the most common type of blood clot that occurs in arteries of APS patients.

A DVT is a clot that forms in the vein in an extremity, usually in the leg. Symptoms of a DVT include swelling, pain, and/or redness in the affected extremity. People are more predisposed to this type of clot if they are inactive or immobilized for prolonged periods of time, such as on bed rest or on a long airplane trip. A potential complication of a DVT is a pulmonary embolus (PE), which occurs when a clot from a DVT in the leg breaks off and travels to the lung, where it becomes trapped. Blood flow to that area of the lung becomes blocked, meaning less oxygen is available to go from the lung into the blood for delivery to other organs. Patients with a PE often seek medical attention with shortness of breath and/or chest pain; they often have low oxygen saturation, meaning the oxygen content of their blood falls. Pulmonary embolism can be life-threatening if not treated immediately with blood thinners.

Patients with APS may form clots in the blood vessels of the brain, resulting in a stroke. A blood clot can also form in another part of the body and then travel to an area of the brain where it can get lodged in a small vessel. If it stays there long enough, the brain tissue does not get enough oxygen and may die, causing a stroke. Depending on where in the brain the injury occurs, motor function (movement of an arm or leg), sensation, or language skills may be diminished or lost. In some cases, these deficits can be reversed or improved with anticoagulation; however, the damage may be permanent. Other times the blood clot breaks up or dissolves on its own, and what appears to be a stroke with loss of function completely reverses within minutes or hours. When this occurs, it is called a transient ischemic attack (TIA).

In addition to DVT and stroke, clots can form in any blood vessel (both veins and arteries) throughout the body including the heart, kidneys, liver, spleen, and extremities. Heart attack (myocardial infarction) occurs when a clot forms in one of the coronary arteries, the blood vessels, that supply oxygen to the heart itself. The lack of blood supply to the heart tissue itself can cause the heart muscle to become damaged. The type of problem that can occur in the kidney of APS patients depends on where the clot occurs. If the clot occurs in the vein leading from the kidney, it can cause flank pain and protein leakage in the urine. If enough protein leaks out in the urine, affected patients can develop marked swelling of the ankles and feet. If the clot forms in the artery leading to the kidney, which carries blood under high pressure from the heart directly to kidney, the blood pressure can increase because the blood does not easily flow though the renal artery. Also small arteries of the kidney can be affected with thrombosis (further discussed below).

Another common manifestation of APS is “pregnancy morbidity,” meaning patients may experience certain complications during pregnancy (discussed in Chap. 20).

Non-criteria Manifestation of Antiphospholipid Antibodies

Skin

A classic skin manifestation of APS is called livedo reticularis, which is lacey and mottled skin that gives the appearance of being able to see blood vessels running beneath the skin in a bluish or reddish color. Livedo reticularis is seen on the knees, thighs, and arms; it can become more pronounced depending on the temperature and other factors. At times it can disappear completely. Livedo reticularis is due to the "clogging" in the tiny blood vessels that feed the skin, which is due to the interaction between aPL and the cells that line the inside of the blood vessel. This interaction causes alteration or spasm in such a way as to cause the appearance of livedo reticularis. The skin usually does not get permanently damaged possibly because the clots or spasms are not complete enough to close off the whole vessel. Livedo reticularis can be an important sign that the aPL are present; it is usually sensitive to temperature but not to treatment.

People with aPL are also prone to the development of skin ulcerations or breakdown. These can develop commonly on the shins, particularly around the ankles, in areas that are subject to repeated trauma just from daily activities. Skin ulcers probably form because the blood vessels which supply the skin become more severely "clogged" from the clots. When the blood supply to an area is not good, the skin cannot heal itself appropriately or in a timely manner.

Heart Valve

Antiphospholipid antibodies can lead to mini-clots or "vegetations" on heart valves, usually on the mitral or aortic valve. Often APS patients with abnormalities of the heart valves do not have any symptoms. Many times these mini-clots go unnoticed, and the only evidence that they are present is an abnormal sound (or heart murmur) which can be heard with a stethoscope as the blood flows through these irregularly shaped valves with clot on them. An abnormality may be seen first on an ultrasound test (echocardiogram) of the heart, before the patient is aware of any problem. In some circumstances, parts of these clots can break off the valve and travel to other organs in the body, blocking blood flow to these areas. It can be serious if the clot travels to a place like the brain, causing a stroke. In rare cases, clots on the valve can cause severely leaky valves that worsen heart function, causing "heart failure" which means the heart muscle itself is weakened and fluid backs up in to the lungs. When this occurs, the affected valve usually needs to be surgically replaced as soon as possible.

Lungs

In addition to pulmonary embolism (discussed above), aPL may increase the blood pressure in the lungs leading to a condition called "pulmonary hypertension (PH)" or "pulmonary arterial hypertension (PAH)," which is a chronic disease. Patients

may experience shortness of breath, dizziness, and fatigue. If it is left untreated, it may lead to right heart failure.

Blood Cells

Antiphospholipid antibodies can also affect the blood cells. Platelets are circulating cell fragments in the blood that play an essential role in the clotting system. Too few platelets can lead to excessive bleeding. Some patients with aPL have low platelet counts, called thrombocytopenia. Often the counts are just mildly low and need to be periodically monitored with a simple blood test. Slightly low platelet counts usually do not cause the patient any clinically significant problems. Other patients may have counts that become very low, which is more likely to happen when the body is actively making clots from the antibodies. If the number of platelets goes lower than 50,000 (normal is more than 150,000), there is an increased risk of bleeding (yes, even though the body is clotting in some other places!), and the low platelet count needs to be followed very carefully.

Red blood cells are responsible for carrying oxygen throughout the body and delivering it to all the tissue in the body. Anemia is a word used to describe low red blood cell counts. This can occur when antibodies attach themselves periodically to the outside or "membrane" of a red blood cell. Once it attaches, it can then cause damage to the cell so that it can no longer function and dies. If this happens to enough red cells, the total number of them in the body can go down. When the damage is mild, the person's body is able to keep up with making enough cells to replace the lost ones. If the damage is severe, anemia can cause a patient to feel fatigued and short of breath due to decreased oxygen delivery in the body.

Kidney

Different than the renal artery or vein involvement, the small vessels and filtering parts of the kidney may develop clots (aPL-nephropathy). This is a slowly progressive kidney manifestation of aPL and may even develop despite anticoagulation. The main clinical manifestation of aPL-nephropathy is hypertension first with a relatively small amount of protein in the urine, followed by increased amount of protein in the urine and progressive renal failure.

Other

Some of the other controversial manifestations of aPL include neurologic symptoms not directly related to detectable blood clots. For example, patients with APS often report migraines; however a direct association between aPL and migraine is not well determined. Antiphospholipid antibody-positive patients can develop seizures, cognitive dysfunction, and white matter changes detected on brain magnetic resonance imaging (MRI) imaging.

Catastrophic Antiphospholipid Syndrome

The catastrophic APS (CAPS) is a rare but life-threatening form of APS, which is defined as clots in three or more areas of the body developing simultaneously or in the span of less than 1 week. Definitive diagnosis of CAPS requires microscopic confirmation of small blood vessel clot based on a biopsy. This life-threatening form of APS carries a high mortality rate, estimated around 30–50%, even with treatment. For reasons that are not well understood, the syndrome can be set off by infection, trauma, or sometimes for no apparent reason. Patients with CAPS suddenly make multiple clots simultaneously in various areas of the body such as the brain, kidneys, intestines, and other organs which can cause damage or threaten life, especially if not treated immediately. During this event, the platelets often drop to low levels. When CAPS occurs, patients are often treated in the intensive care unit with a combination of medications. Following a CAPS event, permanent damage may occur; the extent of this damage depends on the type and severity of the organs involved.

What Is the Significance of Antiphospholipid Antibodies in Lupus Patients?

Compared with lupus patients without aPL, lupus patients with aPL have a higher risk of blood clots, pregnancy morbidity, heart valve disease, pulmonary hypertension, livedo reticularis, low platelet counts, anemia, kidney disease, and moderate/severe cognitive impairment. They also have a worse quality of life and higher risk of organ damage.

What Are the Tests Used to Make the Diagnosis of Antiphospholipid Syndrome?

Antiphospholipid antibodies implicated in APS can be measured in a patient's blood via simple blood tests. Three commonly used tests are:

- Lupus anticoagulant test (based on activated partial thromboplastin time [aPTT] and dilute Russell viper venom [dRVVT] tests)
- Anticardiolipin antibody IgG, IgM, and IgA tests
- Anti-β_2-glycoprotein-I antibody IgG, IgM, and IgA tests

There are commonly accepted criteria for the diagnosis of APS, known as the Updated Sapporo APS Classification Criteria. According to these criteria, a patient must (a) have a clinical event, described as either a vascular thrombosis (arterial, venous, and/or superficial blood clot) or pregnancy morbidity *and* (b) meet laboratory criteria based on the blood tests discussed previously. Although the classifica-

tion criteria are meant mainly for research purposes to define groups of patients for clinical studies, they are a useful guide to help clarify the diagnosis in this complex disorder, which has so many different features. The validity of these criteria in diagnosis has been researched and proven.

Healthy individuals can transiently develop low levels of aPL as many infections cause aPL, which is usually transient if the infection clears; however, if the infection is unrecognized (syphilis, mycobacterial disease) or unsuccessfully treated, the antibody persists. Thus, part of the diagnosis of APS requires that the blood tests be persistently positive when checked at least 12 weeks apart.

Some of the blood tests used to detect aPL can quantify the level of aPL. Higher levels of anticardiolipin and anti-β_2-glycoprotein-I antibodies ($\geq$40 U) correspond to an increased risk of an aPL-related event. The subtype of anticardiolipin and anti-β_2-glycoprotein-I antibodies is also important; IgG is clinically more important than IgM, and IgM is clinically more important than IgA. Similarly, patients with a positive lupus anticoagulant test (which does NOT mean that the patient has SLE) are at an increased risk of having a clinical event compared to patients with anticardiolipin antibodies and/or anti-β_2-glycoprotein-I antibodies. Patients who are positive for all three of the aPL blood tests, especially high levels of anticardiolipin and anti-β_2-glycoprotein-I antibodies, seem to have a higher likelihood of clot formation than those with only one or two positive tests. It is important to note that an individual patient's risk of developing clot is multifactorial, based not just on the laboratory tests but also on lifestyle, drug exposure, and medical comorbidities such as cardiovascular disease risk factors (further discussed below).

How to Prevent and Treat Antiphospholipid Syndrome?

Management of aPL-positive patients depends on the individual patient, his or her aPL-related clinical manifestations, and additional medical conditions. Antiphospholipid antibody-positive patients may have no clinical problems; the prevention of the development of new problems, mainly a blood clot, is the most important management strategy in these individuals.

For all patients with positive aPL, whether or not they have had a thrombotic (clotting) event, the first essential step is understanding the risk of thrombosis and eliminating reversible risk factors known to increase risk of clotting (Table 19.3). "Reversible" clotting risk factors are those that a patient can get rid of, in contrast to the clotting risk conferred by the presence of the aPL, which at this time is not reversible. Such reversible risk factors include smoking, oral contraceptive pills, and hormone replacement therapy (the thrombosis is risk also increased in aPL-negative individuals who receive oral contraceptive pills and hormone replacement therapy). In addition, controlling the traditional cardiovascular disease risk factors such as hypertension or diabetes is extremely important. Certain situations, like surgery, prolonged bed rest, long travel, or immobility, also make the formation of blood clots more likely.

Table 19.3 Important points to keep in mind while assessing the risk of thrombosis in antiphospholipid antibody-positive patients*

Do you have a clinically significant antiphospholipid antibody (aPL) profile?
Discuss with your doctor if your antiphospholipid antibody (aPL) profile is clinically significant (persistent versus transient aPL, lupus anticoagulant test positive versus negative, anticardiolipin or anti-ß$_2$-glycoprotein-I tests moderate-to-high positive versus low titer positive). Keep in mind that not every positive laboratory test is clinically significant.
Do you have antiphospholipid syndrome?
Discuss with your doctor if you have an established diagnosis of APS (symptomatic with history of blood clots) or you only have aPL positivity (nonsymptomatic without clinical events). Keep in mind that if a long-term preventive medication (e.g., warfarin, heparin, aspirin) is needed, it should be determined based on APS disease manifestations and other blood clot risk factors.
Do you have another systemic autoimmune disease such as lupus?
Concurrent systemic autoimmune diseases and aPL increase the chances of blood clots. Thus, the optimal control of your systemic autoimmune disease activity is crucial.
Do you smoke?
Smoking increases the risk of blood clots in aPL-positive patients. The solution is obvious: Avoid smoking and participate in smoking cessation counseling programs if you are a smoker.
Are you on birth control pills or hormone replacement therapy?
These pills may contain estrogen. Increased levels of estrogen heighten your chances of developing a clot. Discuss with your doctor whether other forms of contraception can be considered.
Do you have traditional cardiovascular risk factors such as hypertension, diabetes, high cholesterol, obesity, or a sedentary lifestyle?
Discuss with your doctor about the aggressive management of these conditions, exercise regularly, and eat sensibly. Calculate your cardiovascular risk using tools such as the one offered by the American Heart Association.
Do you have a planned surgical procedure requiring prolonged immobility?
Prolonged immobility interrupts normal blood flow and increases the risk of blood clots. Convey your aPL positivity to your physicians involved in your surgeries so that they can take additional blood clot prevention measures before and after your surgery.
Do you have a planned long journey (more than 4–5 h) by plane, train, or car?
The risk of developing clots, particularly deep vein thrombosis (a clot in a vein, particularly from the legs), is considerable during a long journey. It is recommended to walk at least every hour when traveling. Drink plenty of water and limit your alcohol intake. Wiggle your toes or flex your feet while sitting. In addition, a pair of compression stockings could be worn in high-risk patients. It is controversial if heparin treatment before a long journey prevents blood clots; discuss with your doctor for the final recommendations.
Do you know the early signs of blood clots?
Sudden onset pain, warmth, and swelling of the legs and arms, shortness of breath, chest pain, coughing up blood-streaked sputum, numbness, paralysis or weakness of face or limbs, slurred speech, and visual disturbances are some of the symptoms related to blood clots. You should call your doctor as soon as possible if you develop these symptoms.

*Adapted from Billones I, Erkan D. Blood Clots and aPL-positive patients. Top 10 points to assess and minimize your risk. http://www.hss.edu/conditions_blood-clots-antiphospholipid-antibody-positive-patients.asp

Individuals with persistently positive aPL definitely require close attention during surgical procedures and may often receive low doses of blood-thinning medications to prevent clots, and they should always consult with their doctors before elective surgeries. This is of greatest concern in those patients who are usually on anticoagulation and must stop this treatment before the surgery to avoid bleeding risks. These patients should be watched carefully for signs of clots which can be induced by positioning and intravenous line placements. The risk and benefits of any intravascular procedure, e.g., arterial lines or inferior vena cava (IVC) filter placements, should be evaluated carefully in aPL-positive patients; they are not recommended unless they are absolutely necessary. Additionally, anticoagulation with heparin should begin as soon as possible after the surgery.

All aPL-positive patients should be continuously monitored and counseled by their physicians and/or by special counseling programs for the optimal management of additional clotting risk factors.

Asymptomatic Antiphospholipid Antibody Positivity: How to Prevent a First Clot?

Preventing a clotting event in a patient who has never had a clot before is referred to as "primary thrombosis prevention." Prospective studies have not proven the effectiveness of antiplatelet medication like aspirin for primary prevention; therefore, it is not routinely recommended for asymptomatic aPL-positive patients. However, aspirin is widely used for prevention of cardiovascular events, like heart attacks, in patients with heart disease or cardiovascular risk factors like high blood pressure or diabetes. A patient with aPL and other cardiovascular risk factors such as hypertension or diabetes may be instructed by his or her physician to take a daily low-dose aspirin; we recommend that general population guidelines should be followed to decide about low-dose aspirin treatment. In addition to the elimination of reversible blood clot risk factors, the ideal strategy is a risk-stratified approach to treatment, looking at an individual patient's medical history and risk factors to devise a treatment plan. Please refer to the "Ongoing Research" section below for further discussion.

Antiphospholipid Syndrome: How to Treat an Acute Blood Clot and How to Prevent the Recurrence of a Blood Clot?

The mainstay pharmacologic treatments of APS are medications which thin the blood therefore making clotting less likely. This can be aspirin, or anticoagulants (such as warfarin and heparin), or a combination of the both, depending on the

Table 19.4 Major food and drug interactions that affect warfarin metabolism

Decrease INR (inhibition)	Increase INR (potentiation)
Food	
Leafy greens (spinach, kale, escarole, turnip, or mustard greens)	
Broccoli, cauliflower, cabbage	Excessive alcohol (>3 drinks daily)
Avocado	Fish oil
Endive	Acetaminophen
Canola, soybean oil	Grapefruit juice, cranberries
Drug	
Coenzyme Q10	Antibiotics such as ciprofloxacin, erythromycin, metronidazole, fluconazole
Carbamazepine	
Ginseng	

patient. These medications are routinely used in aPL-positive patients with a history of a blood clot or in an aPL-positive pregnant woman with a history of miscarriages. Obviously, a potential complication of anticoagulation is bleeding, when the blood becomes too thin. Physicians must balance the risk of clotting and the risk of bleeding in each individual APS patient to determine the appropriate selection and duration of anticoagulation treatment.

When a blood clot develops, an intravenous form of anticoagulation called intravenous (IV) heparin is usually started first because it can thin the blood faster than pills can. (Heparin can also be given by injection under the skin.) Once the blood is sufficiently thin, usually the switch is made to the oral, pill form, warfarin (also known as Coumadin® among others). The heparin and the warfarin are often overlapped for a few days until the pills have had a few days to build up in the body and cause the blood to stay thin enough once the heparin is stopped. Once the patient is taking warfarin, it needs to be followed very closely with blood tests on an ongoing basis, and adjustment in the dose often needs to be made, sometimes as often as weekly. The test used to monitor the thinness of the blood is known as the international normalized ratio or INR. The goal of INR will be determined based on an individual's history. In most cases, the recommended INR will be between two and three. Many foods and medications can cause the INR level to increase or decrease (Table 19.4), so it is crucial that patients always let all healthcare providers know that they are taking warfarin.

The length of anticoagulation treatment depends on the individual patient and the circumstances surrounding her thrombotic event. In the vast majority of APS patients with previous clots, the recommendation is for lifelong anticoagulation with warfarin.

How to Prevent Recurrence of Pregnancy Loss?

Please refer to Chap. 20.

How to Treat the Non-criteria Manifestations of Antiphospholipid Antibodies?

Treatment for the non-criteria manifestations of aPL, i.e., symptoms other than blood clots and pregnancy morbidity, depends on the individual patient and clinical circumstance.

Livedo reticularis does not need any treatment. Skin ulcers are usually treated with topical wound care. Sometimes systemic anticoagulation is necessary to promote healing. It is important for APS patients to avoid traumas which can allow these ulcers to form and to keep the areas clean once an ulcer appears to help encourage healing. Once the treatment with blood thinners is initiated, these ulcers may heal because the blood flow to the skin improves and the skin is healthy enough to repair itself again. More often, immunosuppressive treatment may be required in patients with skin ulcers resistant to blood thinners.

Very low platelet counts are rare but can happen and need to be treated aggressively, usually with corticosteroids and/or intravenous immunoglobulin (IVIG). If the number of platelets falls to dangerously low levels where the patient is at risk for spontaneous bleeding, they are given back to the patient in the form of a platelet transfusion. Likewise, if a patient has significant anemia, treatment with steroids is usually indicated. On rare occasions when the red cells get destroyed too quickly or when a person is too sick to keep up with making new replacement red blood cells, transfusions can be utilized. Also, if a patient has profound anemia and a lot of symptoms such as shortness of breath, fatigue, or chest pain, a red blood cell transfusion is usually given.

The benefit of antiplatelet agents such as aspirin or blood thinners such as warfarin for aPL-related heart valve disease (vegetations) and small vessel kidney disease (aPL-nephropathy) is not well demonstrated. Both conditions are slowly progressive. An immunosuppressive approach by rituximab was studied in patients with non-criteria manifestations, and it suggests that rituximab may be effective in controlling some non-criteria manifestations of APS. The role of direct oral anticoagulants and other immunosuppressive agents is under investigation. Please refer to the "Ongoing Research" section below for further discussion.

How to Treat Catastrophic Antiphospholipid Syndrome?

Catastrophic APS is a rare and extremely serious manifestation of APS. Because of how sick patients with CAPS can be and the high mortality rate, a combination of therapies is often employed. The best outcomes in CAPS are usually achieved with the combination of anticoagulation (usually heparin in the acute setting), corticosteroids, and IVIG and/or plasma exchange.

Intravenous immunoglobulin consists of proteins donated from a large number of different people. It is administered intravenously, similar to a blood transfusion. Why this treatment is helpful for patients with APS is not well understood. It may

have a role in helping to neutralize antibodies or prevent the formation of new antibodies. In general, it is only used for the most serious cases during CAPS.

Plasma exchange is a procedure during which a patient's plasma, the liquid portion of blood, is removed and replaced by a plasma transfusion from a healthy donor. Antiphospholipid antibodies are found in the plasma; thus, by removing the patient's plasma and exchanging it for normal plasma, the disease-causing autoantibodies are removed. This is a non-specific treatment whereby all of the antibodies and proteins circulating in the patient's plasma (not just the problematic aPL) are removed.

Cyclophosphamide, an immunosuppressive drug, may be helpful in patients who also have lupus and experience a lupus flare in addition to CAPS. Also, rituximab, another immunosuppressive drug that targets the inflammatory cells that secrete antiphospholipid antibodies, has been used in a limited number of CAPS patients, especially in those with low platelet counts.

Prevention of Blood Clots During Surgical Procedures

Surgery is a major risk factor for the formation of blood clots in everyone due to surgical damage to tissue and blood vessels and immobilization during and after surgery. Antiphospholipid antibody-positive patients are at higher risk for blood clots compared to the general population.

Many APS patients are on long-term blood-thinning medication such as warfarin. This creates a challenge for surgery because warfarin can take several days to wear off. Doctors will often use a strategy called "bridging" wherein a long-acting warfarin is replaced with a short-acting medication such as heparin around the time of surgery. For APS patients on long-term warfarin, the ultimate goal is to keep the blood thin for as long as possible to protect against clots and then to bring the blood to a normal clotting level temporarily during the surgery to prevent blood loss. It is very important to minimize the time spent off blood-thinning medications, so warfarin and/or heparin should be restarted as soon as it is safe to do so after surgery.

Most patients who take aspirin stop the medication 1 week before surgery; however, depending on the type of surgery, aPL-positive and/or APS patients may be advised to continue aspirin before surgical procedures. Discontinuation of other blood thinners such as direct oral anticoagulants, clopidogrel, or dipyridamole can be decided depending on the medical condition of the patient and the type of surgery planned.

Ongoing Antiphospholipid Syndrome Research

There are many other therapies that are currently under investigation for prevention of clots in patients with aPL. Many of these therapies are known as "immunomodulatory therapies," which means that they interact with one's immune system with the

goal of decreasing or eliminating the production of the aPL. Some of the potential immunomodulatory approaches include:

Hydroxychloroquine (Plaquenil®) is an antimalarial drug which is used to treat SLE and some other types of arthritis. Hydroxychloroquine has anti-inflammatory effects and also inhibits platelet aggregation, which is a key step in blood clot formation. There is evidence to suggest that this drug may help reduce the clot-forming properties of aPL in mouse models and it can also decrease the risk of blood clots in SLE patients. Studies looking at the efficacy of this medication in primary and secondary clot prevention are ongoing; hydroxychloroquine can be considered in difficult-to-treat APS patients.

Statins are a class of medications typically used to lower cholesterol levels. There is emerging evidence that these medications have anti-inflammatory effects on various cells in the body. Some studies in mouse models suggested that statins can decrease clot size through interactions with the clotting cascade. In APS patients, statins decrease the level of proteins involved in inflammation and blood clots; however there are no studies demonstrating that statins decrease the risk of blood clots in aPL-positive patients. Thus, currently, there is no definitive evidence that statins prevent clots in APS patients and clinical studies are needed. Like warfarin, this class of medications should be avoided in pregnant patients as they can cause birth defects.

Rituximab is an infusion medication that targets B cells, which are responsible for making antibodies. Rituximab is used in many autoimmune conditions (including rheumatoid arthritis and vasculitis) to decrease antibody production. It has been used to treat immune-mediated anemia and thrombocytopenia in APS patients with anecdotal success; there are limited data that support the use of rituximab in APS patients with hematologic and microthrombotic manifestations.

Complement proteins are a number of small proteins that work together to as part of the body's immune system. Studies in mouse models have shown that activation of certain complement proteins seems to be implicated in pregnancy complications. Furthermore, mice genetically engineered to lack certain complement proteins were less likely to develop aPL-related complications. These findings have led to the hypothesis that medications that inhibit or block certain of these complement proteins may be therapeutic in APS.

Based on a recent study, blocking "mammalian target of rapamycin complex" (mTOR) pathway, which leads to inflammation of the cells located on the inner layer of the artery or vein walls, can be effective in preventing aPL-related clinical problems. In a small cohort of renal transplantation recipients, investigators observed that patients receiving an mTOR-blocking drug (already available for other indications) developed significantly less clinical problems. Thus, mTOR pathway blockade is a promising target in APS; however further studies are needed to better clarify the potential beneficial effect of this immunosuppressive agent for the treatment of aPL-nephropathy or other aPL manifestations.

Direct oral anticoagulants (DOAC), e.g., rivaroxaban, apixaban, edoxaban, and dabigatran, are relatively new agents approved for blood clot prevention and treatment in general population. The advantages of these agents include fixed-dosing regimen, no diet restrictions, fewer drug interactions, and no blood monitoring.

Pending the results of the large-scale controlled clinical studies in APS patients, the role of DOACs in the management of APS patients is unknown. Our recommendation is not to use these agents until further studies are available.

Conclusion

Although the current knowledge about APS has been expanding rapidly, much work still needs to be done in terms of working out why clots happen and the best treatments for different types of patients.

The purpose of this chapter was to help patients understand the present knowledge and research on this syndrome. Some of the frequently asked questions by patients are summarized in Table 19.5. However, one should keep in mind that each patient has a different clinical presentation and your doctor is the best person to discuss your questions.

Table 19.5 Questions frequently asked by antiphospholipid syndrome (APS) patients

Q: Is APS contagious?
A: Definitely not! Although we do not understand how people get the antibodies, it is not spread like an infection.
Q: If I don't have lupus now, am I more likely to develop it later?
A: No. Most people with both diseases develop them at the same time.
Q: If I have a child, what are the risks of the baby having the same problem?
A: Although the hereditary aspects of this disease are not fully worked out, it is not currently believed to be directly inherited. However, it does seem to run in families. It is rare that someone else in the same family would have the antibody.
Q: Are there things which can increase the likelihood of getting a clot?
A: Yes, inactivity – especially for long periods, long plane trips, smoking, use of oral contraceptive pills, and a history of high blood pressure or high cholesterol.
Q: Does APS cause all blood clots?
A: No there are other causes of blood clots. Certain diseases such as cancer or blood disorders can make a person more susceptible to forming clots. There are a number of other inherited clotting problems such as factor V Leiden mutation, deficiency of protein C or protein S, to name a few.
Q: Does APS cause all miscarriages?
A: No. Many more pregnancy losses are because of genetic abnormalities early on. Later on, problems with the blood vessels in the placenta can be a cause.
Q: How long will I need treatment for APS?
A: In those patients who have had clots in the past, anticoagulation would likely be lifelong, though this depends on the individual patient and the circumstances surrounding the clot.
Q: Why is my syphilis test positive if I don't have it?
A: This is confusing. The reason that the test for syphilis is positive has to do with the antibodies reacting to the way the test is done. The antibodies bind to the lipids in the test and make it come out positive. This does not mean that you have syphilis and this is called "false-positive" syphilis test.

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Chapter 20
Obstetric Antiphospholipid Syndrome: What Should Patients Know?

Lisa R. Sammaritano and Roger Abramino Levy

Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the production of antiphospholipid antibodies (aPL) resulting in clinical complications such as blood clots and pregnancy complications. Obstetric antiphospholipid syndrome (OB-APS) defines the subset of APS patients who have the associated pregnancy complications. Detailed information about APS- and aPL-related other clinical problems can be found in Chap. 19.

Definitions

The terminology used in describing APS can be confusing. There is an important difference between the terms "antiphospholipid antibody" (aPL) and "antiphospholipid syndrome" (APS). A positive aPL test alone, even in high levels, does not mean that one has the syndrome: APS is defined by a positive aPL (with persistent moderate or high titers) test in the setting of the typical associated clinical complications. Antiphospholipid antibody-positive patients may be asymptomatic, i.e., have no history of blood clots, pregnancy complications, or other atypical complications, may have APS with only a history of pregnancy complications, or may have APS with blood clot formation (thrombosis) with or without pregnancy complications.

A further confusing aspect of aPL terminology is that there are several antibodies with different names that belong in this category. The most commonly tested aPL, and the ones used to make the diagnosis of APS, are anticardiolipin antibody (aCL), anti-β_2 glycoprotein-I antibody (aβ_2GPI), and lupus anticoagulant (LA).

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Table 20.1 Criteria for classification of obstetric antiphospholipid syndrome

Laboratory criteria	Persistent high titer IgG or IgM anticardiolipin antibody Persistent high titer IgG or IgM anti-β_2 glycoprotein-I antibody Persistently positive lupus anticoagulant
Clinical criteria	Pregnancy loss: ≥3 losses before 10 weeks Pregnancy loss: ≥1 loss after 10 weeks Early delivery (≤34 weeks) due to preeclampsia, intrauterine growth restriction, or fetal distress

Must have one laboratory and one clinical criterion to meet criteria

Both the level of positivity (titer) and the specific type of aPL are meaningful and can be helpful when trying to estimate the risk of complications. A clinically significant aPL test result means that the patient's blood has tested positive at least twice (with a minimum of three months between tests) for one or more of the commonly accepted aPL tests. For aCL and aβ_2GPI, it means that certain isotypes (specific forms of the antibodies) are present at predetermined levels: IgG or IgM isotypes at a level of greater than or equal to 40 units. Any positive LA test is considered significant. Especially in cases of pregnancy complications, other aPL tests may be ordered from commercial or research laboratories, e.g., antiphosphatidylserine or antiphosphatidylethanolamine antibodies, in the hope of shedding light on a patient's pregnancy problems. These are considered "non-criteria" aPL tests: their significance is uncertain, and interpreting these results is often challenging. Lower levels of any aPL are usually clinically less important although may still be associated with complications. Patients who are positive for the LA test have a higher likelihood of aPL-related pregnancy complications.

Obstetric APS refers to the association of pregnancy complications with aPL. Patients with clear-cut OB-APS must meet specific criteria that include pregnancy complications such as preeclampsia, fetal growth restriction and prematurity, recurrent early losses, or a late loss (Table 20.1). A positive aPL test is a major risk factor for pregnancy loss and other poor pregnancy outcomes, especially when present in association with systemic lupus erythematosus (SLE). In trying to decide whether a patient's poor obstetrical history is due to aPL, other causes of pregnancy loss must be ruled out. When considering a diagnosis of OB-APS, all three aPL (LA, aCL, and aβ_2GPI) should be tested.

Risk Factors for Poor Pregnancy Outcomes in Antiphospholipid Antibody-Positive Patients

A summary of risk factors for poor pregnancy outcomes is shown in Table 20.2. In general, pregnancy risk is greatest for those with positive LA (or, in some studies, for "triple aPL-positive" patients described as positive LA combined with aCL and aβ_2GPI). In the PROMISSE study (predictors of pregnancy outcome: biomarkers in

Table 20.2 Assessing risk for pregnancy complications in patients with antiphospholipid antibodies (aPL)

Risk factor	Details
Antiphospholipid type and titer	Positive lupus anticoagulant Triple positive aPL
Other prothrombotic risk factors	History of thrombosis Systemic lupus erythematosus
Additional risk factors	History of prior pregnancy complications Low complement levels Obesity, smoking, hyperlipidemia
Treatment adherence	Poor adherence

APS and SLE), a study that enrolled women with SLE, aPL, and healthy controls, and followed these women through their pregnancies, patients who were positive for LA had a 12-times greater risk of pregnancy complications (including pregnancy loss, preterm delivery, and other associated complications) than those who were negative. Other risk factors identified in the PROMISSE study included younger age at time of pregnancy, a history of blood clots, and having a diagnosis of SLE in addition to positive aPL.

Potential Complications During Pregnancy in Antiphospholipid Antibody-Positive Patients

Although APS patients are more likely to develop pregnancy complications than women in the general population, current management of planned pregnancies allows the majority of women with APS to deliver healthy babies. More than 80% will have live newborns, and approximately 60% will not have any pregnancy complications. Antiphospholipid antibody-related concerns in aPL-positive pregnant patients include maternal and fetal/neonatal complications.

Maternal Complications

Possible maternal complications associated with OB-APS include pregnancy loss, preterm delivery, preeclampsia, eclampsia, HELLP syndrome (all defined below), and development of blood clots in the mother.

Pregnancy loss may occur early (between conception and week 9 of gestation, generally before detection of fetal heartbeat) or later in the pregnancy (between week 10 of gestation and delivery, generally after detection). For a woman with a history of pregnancy loss, treatment during a subsequent pregnancy can improve the likelihood of a successful outcome (see below).

Preterm delivery is defined as delivery before 37 weeks of gestation. In OB-APS, preterm delivery may occur earlier as a result of preeclampsia (high blood pressure and protein in the urine after 20 weeks of gestation), eclampsia (a severe form of preeclampsia that can cause seizures and coma in the mother), or placental insufficiency (alternations in fetal well-being due to problems with the placenta). HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) is a variant of severe preeclampsia and is characterized by a specific type of anemia, elevated liver enzymes, and a low platelet count (blood cells that help clotting to occur). HELLP syndrome may be especially severe in patients with APS: it generally occurs earlier (between 25 and 36 weeks) and may predispose to liver damage or other blood clot formation.

Pregnancy causes what is termed a "prothrombotic" state: the elevated estrogen levels alter levels of normal clotting factors and increase the risk of blood clot formation as a result. The risk of a venous clot is increased fivefold during pregnancy in the general population and likely more so in women with aPL.

Neonatal Complications

The most frequent neonatal (or infant) complications are prematurity and small size for gestational age. Prematurity is most common in patients who have both APS and SLE. Transfer of aPL across the placenta has been shown, but blood clots in the fetus or infant are rare and have been reported in only 21 infants, many of whom had additional risk factors such as catheter placement. The maternal antibodies in the infant circulation tend to disappear within the first six months of life. Children of women with APS have been suggested to have a slightly higher risk of developmental disorders, but these studies are limited: the true degree of risk, if any, is not well defined.

Management of Obstetric Antiphospholipid Syndrome

It is important for aPL-positive patients to have a pre-pregnancy evaluation, when possible, with full discussion regarding risks and plans for pregnancy therapy and monitoring. Patients should then be seen by their rheumatologist (and/or hematologist) and obstetrician as soon as pregnancy is confirmed. Throughout the pregnancy, regular visits with the obstetrician and the rheumatologist are essential and will include blood and urine tests, blood pressure measurements, and obstetrical ultrasound examinations. Fetal monitoring may be done in various ways, including "non-stress tests" (heart rate monitoring of the fetus), Doppler studies (ultrasound measurement of blood flow to the fetus), or ultrasound. Some form of fetal monitoring is part of the routine follow-up in the third trimester for aPL-positive women.

Patients should be familiar with symptoms that may indicate aPL-related complications and that require immediate medical attention:

- Early signs of a blood clot: numbness, swelling, or sudden onset of pain in the legs and/or arms as well as shortness of breath, chest pain, coughing blood or blood-streaked mucous, paralysis or weakness of the face or limbs, slurred speech, and visual changes
- Signs of increased protein in the urine: foamy urine or swelling in hands, feet, or face
- Signs of thrombocytopenia (low platelet count): bleeding from the gum/mouth or nose, bloody or dark stool, bloody urine, and bruising or red dots on the skin, usually first seen on the lower legs
- Signs of preeclampsia (that can occur after 22–24 weeks): high blood pressure, abdominal pain, nausea, vomiting, headache, and change in vision

In addition to being ready to take prompt action should complications occur, APS patients should arrange to deliver at a hospital with a neonatal intensive care unit and other advanced facilities to provide specialized care for APS patients and their babies if needed.

If the mother and the baby are healthy at the time of labor, vaginal delivery is usual and preferable for APS patients like in the general population. However, if the mother and/or baby are under stress, or in the event of preterm labor, a Caesarian section might be the safest and fastest method of delivery. The potential delivery options as well as the management of medications during the delivery should be discussed with the patient's physicians in advance; ultimately, the delivery method is determined by the fetal position and conditions as seen by the obstetrician. There are special considerations if the patient is on anticoagulant medication, including low-molecular-weight heparin and aspirin.

Medical Treatment During Pregnancy

Medical treatment is usually started when the pregnancy is confirmed. In addition to medications such as aspirin and the blood-thinning medication heparin, calcium and vitamin D supplements may also be prescribed to try to reduce the loss of bone mass that can lead to osteoporosis (reduced bone strength), an uncommon complication associated with heparin use.

If a woman becomes pregnant when already on warfarin for a history of blood clots, the warfarin is stopped as soon as pregnancy is suspected or confirmed, because it can cause congenital abnormalities in the fetus especially if given between weeks 6 and 12. Warfarin is changed to heparin (usually low-molecular-weight heparin), a blood-thinning medication given by self-injection. When pregnancy is planned, some patients may prefer to transition ahead of time to low-molecular-weight heparin (prior to trying to conceive) to decrease the risk of inadvertent warfarin exposure.

Women with aPL and a history of fetal loss are usually treated with the combination of heparin and low-dose aspirin. The precise dose of low-molecular-weight heparin depends upon the patient's previous APS manifestation. Women

who have had previous pregnancy complications only (without a history of blood clots) are typically given low-molecular-weight heparin at a lower (prophylactic) dose. Pregnant women who have a history of a previous blood clot and who were previously on warfarin receive a higher (therapeutic) dose twice a day and are determined by the patient's weight. Statistical analysis of the few controlled treatment studies of low-dose aspirin and heparin has confirmed benefit of this combination therapy. Unfractionated heparin can also be used but differs from the low-molecular-weight type in that the dose is determined more exactly by the patient's weight and the dose must be adjusted (and usually changes in the course of pregnancy) by measuring the blood-thinning effect on serial blood tests. Anticoagulation is recommended to be continued for 6–12 weeks after delivery as the risk of blood clots is increased in aPL-positive patients during the postpartum period. When oral anticoagulation with warfarin is restarted after delivery, it must begin with heparin; in order to avoid complications, the injections can only be stopped when the target international normalized ratio (INR) is reached.

If patients have recurrent pregnancy losses on aspirin and heparin, intravenous immunoglobulin (IVIG) may be added based on several successful case reports; although a single small controlled treatment trial did not show a benefit in a low-risk group of patients, larger controlled trials of high-risk aPL patients remain to be performed. Several recent unconfirmed preliminary reports have suggested a possible beneficial effect of the antimalarial medication hydroxychloroquine on OB-APS pregnancy outcome even in the absence of underlying SLE. Hydroxychloroquine is safe for the mother and the developing fetus during pregnancy and can also be used during lactation.

There are no strong data to support treatment of pregnant patients with asymptomatic aPL (that is, no history of blood clots or pregnancy complications), but low-dose aspirin is often used for patients during pregnancy with high-risk (strongly positive) antibody results. Low-dose aspirin may be reasonable for patients with other risk factors for preeclampsia such as SLE, high blood pressure, or kidney problems, since low-dose aspirin has been suggested to decrease risk of preeclampsia in women with risk factors.

Postpartum Care

After delivery, APS patients should follow up regularly with their rheumatologist to monitor their disease. Subcutaneous injections of heparin are recommended for 6–12 weeks after delivery to prevent blood clots in those women who are not returning to warfarin treatment. Special precautions such as elastic compression stockings and early mobilization are important for patients with a history of blood clots and for those who have had Cesarean sections.

Despite limited data, women with purely obstetric APS are usually treated with low-dose aspirin in the long term after pregnancy in addition to recommendations to controlling additional risk factors for blood clot formation.

Medication Safety in Pregnancy and Breastfeeding

Aspirin

No congenital anomalies related to low-dose aspirin use have been reported in humans. High (but not low)-dose aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) can cause premature closure of the fetal ductus arteriosus (a bypass blood vessel in the fetus) in the third trimester, which can lead to heart and lung damage in the infant. High-dose aspirin or NSAIDs should be discontinued by 30 weeks of gestation. Aspirin and ibuprofen may be taken during breastfeeding as a very little amount is transferred to the infant.

Warfarin

While warfarin is contraindicated during pregnancy because of its teratogenicity, it is permitted during breastfeeding. Women who have been on chronic warfarin therapy can switch back to warfarin from heparin in the postpartum period, using both until the INR target is reached.

Heparin (Unfractionated or Low-Molecular-Weight)

Heparin and LMWH are compatible with both pregnancy and lactation. Due to their molecular size, these drugs do not cross the placenta or transfer into the breast milk.

Hydroxychloroquine

Hydroxychloroquine may be safely taken during pregnancy and breastfeeding: studies suggest no negative effects on offspring. It is recommended for pregnant lupus patients but has not yet been formally studied in APS.

Intravenous Immunoglobulin, Prednisone, and Immunosuppressive Medications

There are limited reports on the safety of IVIG during pregnancy, but no cases of congenital anomalies have been reported, and it is felt to be compatible with pregnancy and lactation. Of note, patients with APS and other autoimmune diseases such

as SLE may be on other immunosuppressive medications during pregnancy and while breastfeeding. Prednisone may be safely taken during pregnancy and breastfeeding; if the daily dose of prednisone is high (20–50 mg daily), the mother should breastfeed, take the medication, and wait for four hours after taking the medication before nursing the baby again. Patients may not take cyclophosphamide, methotrexate, or mycophenolate mofetil during pregnancy or breastfeeding; they should discuss the risks and benefits with their physicians if they are on azathioprine, tacrolimus, or cyclosporine, although in general, these medications appear to be low risk for pregnancy.

Calcium and Vitamin D

Calcium and vitamin D are safe at usual dosages during pregnancy.

Contraception

Birth control options should be discussed with both the gynecologist and the rheumatologist. Breastfeeding is not a reliable method of birth control, and estrogen-containing birth control (whether in the form of a pill, patch, or vaginal ring) should *never* be used by aPL-positive patients since it increases the risk of blood clots.

Long-acting reversible contraceptives such as intrauterine devices (IUDs) or subdermal (under the skin) implants are the most effective forms of contraception. Intrauterine devices generally contain either progesterone (levonorgestrel) or copper and may be safely used in teenagers and women who have not yet been pregnant. In general, progesterone IUDs lessen monthly vaginal bleeding – a potential advantage for women on warfarin – while copper-containing IUDs tend to worsen monthly blood loss. Progesterone-only contraceptives represent a safe and effective option for aPL-positive patients, either as a progesterone-only pill, IUD, or implant and – with the possible exception of depomedroxyprogesterone acetate (DMPA) intramuscular injection – do not increase the risk of blood clots. Emergency contraception (e.g., the morning after pill) can be safely used by aPL-positive women.

Fertility Issues

The effect of aPL on fertility has been controversial, with concern in the past that aPL may interfere with implantation of the fertilized egg, particularly after in vitro fertilization (IVF). However, the Practice Committee of the American Society for Reproductive Medicine has released guidelines based on extensive literature analysis stating that there is no indication to check aPL as part of a fertility work-up or to treat aPL-positive women for the purpose of improving IVF cycle outcome.

Patients with aPL may undergo assisted reproduction techniques, including IVF. Ovarian hyperstimulation syndrome (OHSS) is a rare complication of IVF due to very high estrogen levels that may increase risk for blood clots and kidney problems but is very rare. Blood clots in aPL-positive patients undergoing IVF are uncommon, but most reported patients have been treated prophylactically with anticoagulants of some sort. Prophylactic anticoagulation during IVF should be considered in asymptomatic patients with high-risk aPL profiles and is mandatory for patients with history of blood clots.

Conclusion

Pregnancy in patients with primary APS or APS related to SLE is considered high risk and should be managed by a multi-professional team. Planning ahead and adjustment of medications are crucial for better fetal and maternal outcomes and are good adherence to treatment and life-style recommendations.

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