“Black swan in the kidney”: Renal involvement in the antiphospholipid antibody syndrome

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“Black swan in the kidney”: Renal involvement in the antiphospholipid antibody syndrome. The antiphospholipid antibody syndrome (APS) describes a clinical entity with recurrent thrombosis, fetal loss, thrombocytopenia in the presence of lupus anticoagulant and/or antibodies to cardiolipin. These antibodies may be associated with connective tissue diseases such as systemic lupus erythematosus (secondary APS) or be found in isolation (primary APS). Renal syndromes increasingly being reported in association with these antibodies include thrombotic microangiopathy, renal vein thrombosis, renal infarction, renal artery stenosis and/or malignant hypertension, increased allograft vascular thrombosis, and reduced survival of renal allografts. Although much has been understood concerning the biology of these antibodies and the pathogenesis of thrombosis, the optimal therapy remains to be elucidated. This article presents a historical review of the renal involvement in the antiphospholipid syndrome and discusses therapeutic options. Further research is needed.

It has been more than 15 years since Harris, Hughes and others described the clinical features of the antiphospholipid antibody syndrome (APS) [1–3]. Since then, the syndrome originally scorned as a “black swan” or a laboratory error has evolved as a distinct clinical entity [4] that is associated with protean clinical manifestations such as arterial and venous thrombosis, recurrent abortions, and thrombocytopenia. Renal manifestations of this syndrome have recently received more attention as the kidney appears to be a major target organ in both primary and secondary APS. Although more prevalent in patients with systemic lupus erythematosus (SLE), antiphospholipid antibodies (APAs) occur in patients without other manifestations of autoimmune disease, the so-called “primary” APS. The preliminary classification and criteria for diagnosis of APS were published in 1999 [5] and were recently validated [6]. In this review, we provide an overview of the mechanism of thrombosis in APS, the spectrum of renal abnormalities encountered with this syndrome, and the available treatment options.

BIOLOGY OF ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies are a heterogeneous group of immunoglobulins directed against negatively charged phospholipids (such as phosphatidyl serine, or cardiolipin), protein-phospholipid complexes, or plasma proteins such as beta 2-glycoprotein I (β2-GPI). Phospholipids play a critical role in the normal control of the coagulation as depicted in Figure 1. For example, activated protein C inactivates factors Va and factor VIIIa in the presence of phospholipids and factor S, thus inhibiting the generation of thrombin [7]. APA include lupus anticoagulant and anticardiolipin antibodies. The lupus anticoagulant is detected in coagulation assays as it slows the rate of thrombin generation by perturbing interactions that require phospholipid, thus acting as an anticoagulant. The clue to the presence of a lupus anticoagulant is often an increased partial thromboplastin time (PTT) that fails to correct on mixing studies with normal plasma. The anticardiolipin antibodies, in contrast, are recognized by the ability to bind anionic phospholipids in solid-phase immunoassays. Thus, anticardiolipin and lupus anticoagulant are distinct but complementary tests for APAs. The genesis of APAs may involve both autoimmune and alloimmune reactions. Alloimmune APAs may be observed following an infection such as hepatitis C, Lyme disease [8–10], human immunodeficiency virus infection (HIV), or in association with lymphoproliferative disease, and appear to have a weaker association with thrombotic complications [8, 9]. Autoimmune APAs bind phospholipid epitopes in the presence of a cofactor and have more consistently been associated with hypercoagulability. Vascular thrombosis represents the clinical hallmark of APS and both arteries and veins may be
affected. Thrombosis may affect deep leg veins, as well as those of the skin, lungs, kidney, central nervous system, and placenta. Thrombocytopenia is quite common. While the association between these APAs and thrombosis is now well established, the unresolved central issue in both primary and secondary APS is how antibodies against phospholipids lead to increased tendency to thrombosis. In the case of lupus anticoagulant, this effect appears paradoxical since an anticoagulant would be expected to cause bleeding, not thrombosis. Phospholipid-dependent coagulation reactions include the following reactions: (1) tissue factor-factor VII (VIIa) mediated activation of factors X and IX; (2) factor IXa and factor VIIIa activation of factor X to factor Xa; and (3) factor Xa and factor Va activation of prothrombin to thrombin (see Fig. 1). The lupus anticoagulant appears to act by limiting the amount of phospholipid available to support these reactions, and thus prolonging the coagulation times. A major advance in the understanding of the thrombophilic effect of these antibodies occurred when two groups led by McNeil et al [11] and Galli et al [12] independently observed that the major antigen (cofactor) for APA is a plasma protein, apolipoprotein H, or β2-GPI. This protein, a glycosylated single-chain plasma protein composed of 326 amino acids with a molecular weight of 50 kD, is a weak anticoagulant that binds to anionic phospholipids and inhibits the contact phase of coagulation and the activity of platelet prothrombinase. The exact nature of these epitope(s) remains uncertain. Data from this laboratory however, show that though β2-GPI increases antibody binding to phospholipid, it is not an absolute prerequisite for binding [13]. Other data suggest that monoclonal antibodies with lupus anticoagulant activity can increase the binding of β2-GPI to phospholipid [14]. This process then interferes with essential phospholipid-dependent steps in coagulation, especially the assembly of the tenase and prothrombinase complexes on anionic phospholipids. Consistent with these observations, thrombosis has been induced experimentally by immunoglobulins (Ig) IgG, IgM, and IgA from patients with APS [15]. Proteins besides β2-GPI have also been implicated in antiphospholipid antibody activity. Patients with positive serology for antibodies to β2-GPI often have positive anticardiolipin assays, while serum samples positive for lupus anticoagulant but negative for anticardiolipin appear not to exhibit binding to β2-GPI [16, 17]. Substantial heterogeneity exists among APAs as they react with other hemostatic plasma proteins, with platelets and vascular endothelial cells. Alterations of tissue factor activity [18], as well as the inhibition of activated protein C and protein S by plasma containing APAs, represent potentially thrombophilic changes that have been shown by other investigators. While binding of APAs to platelets appear to be dependent on β2-GPI, other autoantibodies react with the major platelet membrane glycoproteins [19, 20]. The anti-platelet effects of APAs have been suggested to be analogous to that observed in heparin-induced thrombocytopenia [20], as β2-GPI binds to exposed anionic phospholipids on the surface of activated platelets. Platelets circulate in an increased state of activation in APS, but it is unclear if this is a cause or effect of thrombosis. APAs also show substantial reactivity against endothelial cells. [21] In vitro data suggest that this reactivity of APAs against endothelial cells involves both β2-GPI-dependent and independent mechanisms. Since virtually any of the biologic processes that involve or require phospholipids may be affected by the presence of antibodies that bind to the phospholipids (either directly or via cofactors), any proposed APA-mediated effect based on in vitro studies must be evaluated for in vivo relevance. Some of these effects are discussed below and summarized in Table 1.

PATHOGENESIS OF THROMBOSIS IN APS

The precise mechanism by which the presence of APAs induces thrombosis is not completely understood. Experimental and human data suggest that thrombosis induced by APAs may involve changes at the following sites: (1) dysregulation of coagulation; (2) platelet activation; or (3) endothelial injury, disordered transmembrane signaling, and microvasculopathy. These putative pathophysiologic changes are summarized in Table 1. Thus, these antibodies induce a hypercoagulable state, which meets Virchow’s triad for thrombosis (that is, abnormal rheology, tissue injury and abnormal blood constituent(s) for thrombogenesis) [22]. Data from our institution have shown that the thrombogenic effects of these antibodies are not confined to human APA, but have also been shown for antibodies induced in mice [23]. There is substantial evidence that endothelial cell injury/activation plays a role in both in vitro an in vivo models of APS [23–25]. Nakamura et al showed that lupus anticoagulant can induce apoptosis in human umbilical endothelial cells [21]. In their study, annexin V was necessary for induction of apoptosis. Recent data suggest that endothelial cell activation in APS may involve enhanced expression of adhesion molecules such as intercellular cell adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule [VCAM-1], and E selectin [26, 27]. The fact that arteries, veins, and capillaries are potential thrombotic sites in patients with APA reflects the complexity of the underlying pathophysiologic mechanisms. However, thrombotic episodes are generally either venous or arterial in a given patient, but not both. While venous thrombotic events with APAs likely result from perturbations of the protein C/protein S regulatory system [17, 24, 28], platelet activation appears to play a central role in arterial thrombosis in APS. Other poten-
Fig. 1. Control of coagulation showing the role of phospholipids and sites of potential antiphospholipid antibody (APA) action. Three critical steps of the cascade are catalyzed by phospholipids: (1) the tenase complex; (2) the prothrombinase complex; and (3) the protein C activation complexes. Activation of the extrinsic or intrinsic pathway leads to conversion of factor X to Xa. Factor Xa forms a complex with factor Va and prothrombin on a phospholipid template (prothrombinase complex) and results in thrombin generation from prothrombin. Activated protein C inactivates factor Va and factor VIIIa in the presence of protein S and phospholipids. APAs may affect all the pathways indicated with an asterisk, resulting in a procoagulant effect or in a “lupus anticoagulant effect” (by inhibition of conversion of prothrombin into thrombin).

Fig. 2. Kidney biopsy findings in 35-year-old woman with antiphospholipid antibody syndrome (APS) nephropathy and systemic lupus erythematosus (SLE) who developed rapidly progressive renal failure. The biopsy findings of glomerular and arteriolar thrombosis, clearly suggest that APS nephropathy caused the renal failure, rather than lupus nephritis. (A) Arteriole with recent thrombus (arrows) (hematoxylin and eosin stain; magnification ×400). Reprinted from reference [46] with permission. (B) Glomerular capillaries in same patient are filled with fibrin thrombi (hematoxylin and eosin stain; magnification ×200). (C) Glomerular capillaries occluded by agglutinated red blood cells.
Table 1. Pathophysiologic changes contributing to thrombosis in APS

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent activation of coagulation</td>
<td>Increased markers of thrombin generation have been observed; increased levels of plasminogen activator inhibitor-1 (PAI-1)</td>
<td>[8, 13]</td>
</tr>
<tr>
<td>Interference with the anticoagulant effect of β2-GPI</td>
<td>Poor association of levels of β2-GPI with thrombosis; affinity of APA from patients for β2-GPI may be low</td>
<td></td>
</tr>
<tr>
<td>Inhibition of fibrinolysis, activated protein C resistance</td>
<td>Increased clot thickness observed in animal model of APS</td>
<td>[15]</td>
</tr>
<tr>
<td>Increased plasma concentration of tissue factor</td>
<td>APAs enhance endothelial release of tissue factor</td>
<td>[16]</td>
</tr>
<tr>
<td>Endothelial cell injury</td>
<td>Lupus anticoagulants induce apoptosis in human umbilical vein endothelial cells</td>
<td>[21]</td>
</tr>
<tr>
<td>Cellular activation involving platelets, monocytes, endothelial cells</td>
<td>APAs up-regulate expression of adhesion factors ICAM, VCAM</td>
<td>[23–25]</td>
</tr>
<tr>
<td>Inflammation, accelerated atherosclerosis</td>
<td>APAs increase production of arachidonic and metabolites</td>
<td>[26]</td>
</tr>
</tbody>
</table>

Abbreviations are: APS, antiphospholipid antibody syndrome; β2-GPI, beta 2-glycoprotein I; ICAM, intercellular adhesion molecule; VCAM, vascular cellular adhesion molecule.

Table 2. Renal syndromes associated with APAs

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>Glomerular thrombosis (TMA)</td>
<td>[31, 32, 34]</td>
</tr>
<tr>
<td>? worsens outcome</td>
<td></td>
<td>[40]</td>
</tr>
<tr>
<td>Renal infarct and vein thrombosis</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td>Acute renal failure</td>
<td>May be reversible or irreversible</td>
<td></td>
</tr>
<tr>
<td>Renal vein thrombosis</td>
<td></td>
<td>[55, 56]</td>
</tr>
<tr>
<td>Renal artery thrombosis/infarction</td>
<td></td>
<td>[64]</td>
</tr>
<tr>
<td>Pregnancy-related renal failure</td>
<td></td>
<td>[36]</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>With IgA deposits</td>
<td>[53]</td>
</tr>
<tr>
<td>TMA</td>
<td>TTP/HUS</td>
<td>[37, 42, 43]</td>
</tr>
<tr>
<td>Renovascular/malignant hypertension</td>
<td>Renal artery stenosis may be unilateral or bilateral</td>
<td>[68–74]</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Variable from mild to nephrotic</td>
<td>[60]</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>Vascular access thrombosis</td>
<td>[89–92]</td>
</tr>
<tr>
<td></td>
<td>High prevalence in hemodialysis</td>
<td></td>
</tr>
<tr>
<td>Renal transplantation</td>
<td>Graft thrombosis and allograft failure</td>
<td>[93–96, 97, 100, 101]</td>
</tr>
<tr>
<td></td>
<td>APAs may be acquired post-transplant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased morbidity</td>
<td></td>
</tr>
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Abbreviations are: SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy; APA, antiphospholipid antibodies; HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura.

tially thrombogenic effects involve inflammation and atherosclerosis [29]. Taken together, these experimental data suggest that thrombosis in association with APAs involves interference with endogenous anticoagulant systems (such as protein C, annexin V, or vascular anticoagulant alpha), cellular activation (involving platelets, endothelial cells, and leukocytes), persistent activation of coagulation system due to tissue factor activation and inhibition of the antithrombin III pathway, as well as inhibition of fibrinolysis (see Table 1).

RENAL SYNDROMES IN APS

The kidney manifestations of APS may result from thrombosis occurring at any location within the renal vasculature, that is, in the renal artery trunk or branches, intra-parenchymal arteries and arterioles, glomerular capillaries, and the renal veins. The resulting clinical syndromes consists of variable degrees of proteinuria; exacerbation of SLE nephropathy; acute renal failure; systemic hypertension, ranging from mild to accelerated hypertension; renal cortical necrosis; thrombotic microangiopathy; progressive chronic renal failure; as well as thrombosis in patients with renal allografts (Table 2).

ANTIPHOSPHOLIPID ANTIBODIES IN SLE-ASSOCIATED NEPHRITIS

Conley and Hartman initially described the presence of lupus anticoagulant in SLE patients in 1952 [30]. Subsequently, Bowie et al described thrombosis occurring in patients with circulating anticoagulants in a seminal paper in 1963. [31] These observations of the association of APA with glomerular and arterial thrombosis in SLE were extended over the next two decades by Kant et al, who reported a strong association between the presence of lupus anticoagulant and the presence of glomerular thrombi in renal biopsy specimens systematically performed in SLE patients seropositive for lupus anticoagulant [32]. While the precise origin of APAs in SLE is unclear, their presence appears to consistently correlate with the occurrence of renal thrombotic microangiop-
that these observations were based on a study of only SLE, APS nephropathy was observed in 63% of patients. The proportion of SLE patients with both lupus anticoagulant and antiphospholipid antibodies varies from as high as 61 to 91% [32, 33–35] to about 15 to 30% in other series, although not all of them have APS. This wide variation in incidence reflects differences in assay methods and case-mix of patients. The morbidity associated with APAs in SLE has been a subject of considerable controversy [36–39]. While some data show that TMA occurring in SLE patients with APAs was associated with worse prognosis, Naiker et al recently confirmed a high prevalence of APAs in South African patients with lupus nephritis (45%), but found that the presence of these antibodies per se appeared not to change outcome [38], except for an increased incidence of thrombocytopenia and renal vascular thrombosis. These thrombotic events involved large arteries and veins as well as the glomerular and intrarenal arteries and arterioles. However, the study is limited by the fact that these observations were based on a study of only 18 patients. Previously, Kincaid-Smith et al had reported the occurrence of pregnancy-related acute renal failure in 12 women (only two of whom had lupus nephritis) caused by TMA in relation to lupus anticoagulant [36]. It is unclear at present if APA are initiators or accelerators of arterial lesions in TMA, but both roles may be involved. It should be emphasized that the frequent absence of immunoglobulin deposits in vessels involved in TMA suggests that thrombosis may be due to mechanisms other than immune-complex disease. In most series [38–40], more glomerular thrombi are found in those SLE patients with APAs than those without APAs, suggesting an increased morbidity, mostly related to TMA, although these APAs do not affect the World Health Organization’s (WHO) histologic classification. In the same area, it is unclear at this time if these antibodies affect the response to chemotherapy in lupus nephritis; although microvascular lesions of SLE, in general, are considered to be independent risk factors for the progression to end-stage renal disease (ESRD) [41]. An area of on-going controversy relates to the association between the APS-associated lesions (such as glomerular thrombi) and those of lupus nephritis. Miranda et al found no association between the presence of these glomerular thrombi and severity/activity of lupus nephritis [40]. However, only nine of their patients were positive for APAs. These findings support earlier observations by Farrugia et al, who equally found no association between APAs and the type of lupus nephritis lesion [42]. In contrast, Bandari et al suggest from their study of 51 patients, that the presence of anticardiolipin antibody and/or glomerular thrombi portends a worse renal outcome, as patients with APA-positivity had significantly more crescents, more sclerosis, and more glomerular necrosis [43]. This finding does not, however, prove a causal relationship between inflammatory lupus renal lesions and APA positivity. Moreover, Perez-Vaquez et al have suggested that the prevalence of APS in SLE increases with duration of follow-up and on the number of samples tested for APAs [44]. This finding further confounds the interpretation of the relationship between APAs and lesions of lupus nephritis. It is interesting that loss of renal function in SLE patients with APAs can also occur without evidence of lupus nephritis due to APS [45]. In this situation, distinguishing between renal failure in SLE due to nephritis (immune-complex disease) from that due to APS (glomerular thrombosis) can only be accomplished by kidney biopsy [46] (see Figure 2). This distinction is critical as the treatment for each syndrome is different. Aggressive lupus nephritis requires cytotoxic therapy, whereas APS nephropathy benefits from anticoagulation. The nephropathy of APS occurring in the course of SLE is the most common form of secondary APS. In one study of a cohort of 100 patients with SLE, APS nephropathy was observed in 63% of patients with APS (abstract; Daugas E et al, J Am Soc Nephrol 10:A99, 1999). In a recent systematic review of renal biopsies in another 114 patients with SLE, the same investigators studied the lesions of secondary APS nephropathy coexisting with those of lupus nephritis. They found secondary APS nephropathy in 36% of this cohort of SLE patients, even though only 22% of their patients had APS [47]. This higher proportion of APS nephropathy raises the possibility that renal-limited microvascular disease may occur in the course of SLE in some patients. The strengths of this study include a fairly large sample size (114 patients), as well as a strict definition criterion for APA. This study also found greater correlation between lupus anticoagulant positivity and intrarenal thrombosis than was found in patients seropositive for anticardiolipin antibodies. In addition to the TMA lesions seen acutely in APS nephropathy associated with SLE, other lesions such as fibrous intimal hyperplasia, and focal cortical atrophy have also been observed in the affected kidneys [47]. An aggressive vasculopathic state with widespread thrombosis called catastrophic antiphospholipid syndrome (CAPS) may also occur in SLE [46, 48]. This syndrome is described in greater detail in the discussion of primary APS below. Hughson et al reported on the outcome of treatment in three SLE patients with renal TMA and APS [37]. Renal failure and thrombocytopenia resolved in each case following therapy with plasmapheresis, prednisone, and anticoagulation [37]. The association between the presence of APAs in SLE and renal transplant outcomes, as well as other renal complications
such as renal vein thrombosis and renal infarction, is discussed below. In some cases, chronic lesions of APS nephropathy (such as fibrous intimal hyperplasia and focal cortical atrophy) may co-exist with lesions of lupus glomerulopathy [47]. The balance of the evidence at this time favors the possibility that the occurrence of APS nephropathy in patients with lupus nephropathy may increase the risk of progression to ESRD, even though the WHO classification is unaffected. In the largest series published to date [47], these patients had higher creatinine at time of diagnosis, increased interstitial fibrosis, and a higher prevalence of hypertension, as discussed later. No study, to our knowledge, has addressed the relative prevalence of both TMA and large-vessel occlusive disease in SLE patients with or without APS.

**RENAL DISEASE IN PRIMARY APS**

In contrast to the well-described nephropathy associated with SLE and APS, it was not widely appreciated that the primary APS could lead to nephropathy until recently. The studies of the renal lesions in primary APS have enabled investigators to observe the lesions due to APS in pure form [47, 49–54], without the confounding effect of co-existing inflammatory changes. Several patients in these reports presented with acute renal failure (ARF). The precise pathogenesis of ARF in the primary APS has not been clearly delineated, but is likely to be multifactorial. It may result from TMA, renal cortical necrosis, renal infarction, renal vein thrombosis [55, 56], or in the unique aggressive vasculopathy, CAPS [57–63]. This is associated with multi-organ failure and widespread thrombotic diathesis. The renal lesions of the primary APS nephropathy are identical to those seen in other TMAs, including postpartum renal failure and sclerodermal renal crisis, with severe narrowing of the interlobular arteries and afferent arterioles. Predictably, renal ischemia follows these diffuse thromboses. In a few cases, mesangial IgA deposition has been described [53, 54]. Occasionally, thromboses occur in the large renal arteries leading to renal infarcts [64]. The urine sediment typically contains few cells or casts, and the level of proteinuria varies from mild to nephrotic. The histologic lesions associated with primary APS-related nephropathy have been well characterized and include arteriosclerosis (75%); fibrous intimal hyperplasia (75%); tubular thyroidization (75%); arteriolocclusions (68%); TMA (31%), and organizing thrombosis (37%) [49]. Some novel glomerular ultrastructural changes have recently been proposed to be pathognomonic for APS nephropathy [62]. On light microscopy, the silver stains show a combination of glomerular basement membrane wrinkling and reduplication. By electron microscopy, redundant wrinkled segments of basement membrane are accompanied by a “new,” straighter thin basement mem-

**APA IN SYSTEMIC HYPERTENSION**

Hypertension is a common feature of the primary and secondary APS [47, 49]. In the series of primary APS reported by Nochy et al, hypertension was present in 93% of their patients and was sometimes the only clinical sign suggestive of nephropathy [49]. In the secondary APS series reported by Kleinknecht et al, all patients with SLE and APS had severe hypertension with renal insufficiency [67]. Thus, hypertension appears to be a marker of nephropathy in both primary and secondary APS. No study to our knowledge has addressed the prevalence of APAs in an unselected population of patients with essential hypertension. Hypertension in APS may often be severe, with some patients presenting with hypertensive emergencies. Rarely, hypertension associated with APS may be associated with microangiopathic hemolytic anemia. It needs to be emphasized that both microvasculopathy and/or occlusion of the main trunk of the renal artery can contribute to malignant hypertension in the APS. Caccoub et al described five patients with APS complicated by hypertensive crises who all had normal magnetic resonance angiograms of the renal vessels [68]. Three of their patients underwent contrast angiography, which showed distal microangiopathy, but no stenosis or thrombosis of the main renal arteries. High-dose steroids (prednisone, 1.5 mg/kg/day) and anticoagulation led to rapid resolution of hypertensive crises in three of their patients. Five other reports have documented occlusion of main renal arteries leading to renovascular hypertension [68–74]. Most of the patients reported with main renal artery stenosis had had very high titers of APAs, but in one case the titers were moderate [64]. The outcome of renovascular hypertension with APS appears to be good with treatments like anticoagulation, with or without percutaneous balloon angioplasty, leading to recovery of renal function and return to normal blood pressure [68–74]. The contribution of undiag-
nosed APS in cases of ESRD assumed to be due to essential hypertension is unknown. That the renin-angiotensin system appears to be activated in malignant hypertension due to APS is supported by data showing a positive catopril test in those with large vessel renal artery stenosis, as well as positive staining for renin containing cells in some biopsied subjects [49].

APA IN END-STAGE RENAL DISEASE

Data are few and conflicting concerning the prevalence and risk factors for APAs in ESRD. These differences probably depend on the patient mix and assays used for diagnosis. Patients with ESRD have higher frequency of positivity for APAs compared to the general population [75–78].

Moreover, the prevalence of lupus anticoagulant was higher among patients treated by hemodialysis (30%), compared to those treated by conservative (non-dialytic) approach. Subsequently, Sitter, Spannal, and Schiffi reported that the prevalence of APAs were higher in hemodialysis patients compared to those on continuous ambulatory peritoneal dialysis (CAPD) [77]. This higher prevalence in hemodialysis has been confirmed by others [78]. Quereda et al in a prospective study of 138 patients with nephropathy, found that the prevalence of APAs was higher in patients with SLE (34%) than in those with chronic primary glomerulonephritis (9%) and lowest in patients with non-immunologically mediated renal diseases (2.6%) [79]. The origin of APAs in ESRD patients is unclear. It has been postulated that uremia is a form of immunodeficiency in which autoimmunity may develop as a result of altered immune function [80]. Some have suggested that dialysis membranes generate APAs due to incompatibility. In the study by Garcia Morton et al [78], patients dialyzed with cuprohane membranes had a greater incidence of APAs than those dialyzed against more biocompatible membranes. However, in a larger study by Brunet et al, there was no relationship between the APAs and the type of dialyzer used [80]. Other suggested reasons for generation of APAs in ESRD patients include trauma to blood passing through the hemodialysis circuit, as observed in recipients of left ventricular assist devices [81] and induction by microbial agents [82] or their products, such as endotoxins present in dialysate. If this were true, one would expect a correlation between prevalence/titers of APAs and length of time on dialysis. Such a relationship has not been shown at this time. Moreover, not all patients exposed to same dialysate develop APAs. Although the precise mechanisms involved in the genesis of APAs in ESRD are unknown, they appear to mostly β2-GPI-independent [83]. Substantial controversy surrounds the precise risks associated with the presence of APAs in ESRD patients. While some studies suggest that these antibodies are not pathogenic [84–87], other studies have suggested that APAs are associated with hemodialysis vascular access thrombosis [88–91]. Whether both anticardiolipin antibodies and lupus anticoagulants are equipotent in causing recurrent access thrombosis is unclear at this time. Some studies suggest that both lupus anticoagulants and anticardiolipin antibodies are associated with an increased risk of vascular access thrombosis [88, 89], while other studies have verified this relationship only with lupus anticoagulation [86, 90]. Other unresolved issues at this time concerning vascular access thrombosis with APAs include whether rates of occlusion of native arteriovenous fistula thrombosis are identical to those of polytetrafluoroethylene (PTFTE) grafts, or whether APAs cause in-dwelling catheter thrombosis, and if prophylactic anticoagulation would prevent these events. However, on the strength of the epidemiologic and clinical association of these antibodies, it would appear prudent to incorporate APA testing into the diagnostic evaluation of recurrent thrombotic episodes on patients receiving hemodialysis [88–91].

APAS IN RENAL TRANSPLANTATION

In renal transplant patients, the incidence of APAs may also be increased, even in those without SLE [92]. In this study of patients without SLE, a prevalence of 28.1% was documented. Patients with and without APAs did not differ in age, gender, underlying disease, immunosuppressive regimen, serum creatinine concentration, and platelet count. Of these APAs, 65.4% were acquired during the post-transplant period. Interestingly, 19 pre-transplant APAs disappeared in 52.7% of patients in that series after transplant. The genesis of APAs after renal transplant is unclear. Both pre-transplant and post-transplant APAs were associated with thrombosis affecting the dural, femoral, and transplant veins.

In the case of SLE patients with APAs in renal transplantation, their survival is worse than those of SLE renal transplant patients without APAs [93]. Because of this, Joseph, Radhakrishn, and Appel have suggested that patients with SLE who have APAs require attention in the post-transplant period to detect possible thrombotic events and/or consider prophylactic anticoagulation [94]. Stone, Amend, and Criswell studied the clinical events in 96 consecutive patients with SLE after renal transplantation [95]. They observed positivity for APAs in 29.1%. Among these, 60% had clinical events related to APAs, including deaths related to APS (10%); deep venous thrombosis and pulmonary embolism (24%); strokes (16%); renal artery or renal vein thrombosis (16%); and fetal loss (12%) [95]. Although APAs in hepatitis C-positive ESRD patients appear to be non-pathogenic, preliminary data suggest...
this may not be the case in post-repulsion transplantation, as these patients may be at increased risk for allograft TMA [96]. Anecdotal case reports also suggest that patients with primary APS who undergo renal transplantation may also have increased risk for graft failure [97]. How APAs may be acquired after transplantation is curious, but is as yet unexplained. None of these patients were on drugs such as chlorpromazine, hydralazine, or procainamide that can induce these antibodies. It has been suggested that these APAs may be related to post-transplant infection or autoimmune reactions to infections. Consistent with these observations, high levels of APAs are associated with cytomegalovirus infection in unrelated bone marrow and cord blood allogeneic stem cell transplantation [98].

Other studies have addressed the impact of APAs on renal allograft failure [99–101]. In one study Wagen Knecht et al reported significantly more APAs in patients with early renal allograft failure than in patients with functioning grafts. In that study, 57% final cross-matched sera from patients with early nonfunction were positive for IgG, IgM, and IgA APAS [99]. Thus, it is possible that testing for APA may identify certain patients at risk for allograft failure. Biopsies of these failed allograft kidneys from APA-positive patients showed thrombi in nine patients and infarction in five. However, these patients also received OK T3 induction therapy, which may have induced renal allograft thrombosis [102], even though the same OK T3 indication therapy was used in patients with functional allografts. Thus, the impact of immunosuppression in renal allograft thrombosis must be excluded to better quantify the impact of APAs in early allograft failure. However, a role for coagulation anomalies, such as factor V Leiden mutation, deficiencies of protein C and S, as well as lupus anticoagulant in early renal transplant failure, has been suggested by some investigators [99]. Fischereder et al found that thrombophilia represented a 3.5-fold increase in risk for 1-year renal allograft loss [103]. Although the mechanism for early graft failure in APA-positive patients is unclear, perturbations of hemostasis appear plausible. In an immunocytochemical study of failed renal allografts, significantly more depletion of vascular antithrombin and increased fibrin deposition were observed by Tory et al in transplants that failed in the first month than those that failed 2 to 12 months or later after renal transplantation [104]. No differences in cellular infiltrates were observed, thus excluding cellular rejection. This pattern of allograft loss with fibrin deposition in the absence of cellular infiltrates has also been observed in failed cardiac allografts where depletion of vascular tissue plasminogen activator and endothelial cell activation have been implicated [105, 106]. Wagen Knecht et al provide further proof of the role of APA in early renal allograft loss in their report of a case in which they isolated APA from a thrombosed renal allograft [107]. This patient lost two renal allografts in the first month after renal transplantation. The risk of early renal allograft loss in patients with ESRD with APAs who received renal transplants without hemodialysis was 100% in the study of Wagen Knecht et al, suggesting a possible protective role for renal allograft thrombosis by a period of hemodialysis. The reason for the possibly protective effect of a hemodialysis history is unclear, but it is likely that prolonged exposure to heparin with hemodialysis may be responsible. Consistent with these observations, in vitro studies show that heparin can inhibit or neutralize APA binding [108]. Other possible mechanisms of renal allograft dysfunction in APA-positive patients include allograft glomerulonephritis, renal vein thrombosis, and post-transplant ARF [109].

Unresolved questions include whether patients with APAs with normal renal function should serve as kidney donors, in light of possible future renal complications. Recently, a case was reported of a patient who developed ARF requiring hemodialysis due to renal artery occlusion 6 years after donating a kidney to his mother [110]. ARF in this patient was irreversible, and the patient became dialysis-dependent.

MANAGEMENT OF THROMBOSIS IN APS
Diagnostic difficulties
It is clear that APS shows substantial heterogeneity in clinical features, as well as the range of autoantibodies. Accurate diagnosis is critical because of the high risk of recurrent thrombosis. This heterogeneity mandates a comprehensive approach to laboratory testing [111–114], as shown in Table 3. Thus, it is recommended that when APS is suspected, a panel of tests, including lupus anticoagulant, and anticardiolipin antibody, assays be performed.

Lupus anticoagulant assays
The common denominator of all lupus anticoagulant tests is that they detect the inhibition of phospholipid-

### Table 3. Testing for antiphospholipid antibodies

<table>
<thead>
<tr>
<th>Tests for lupus anticoagulants</th>
<th>Criteria</th>
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<tbody>
<tr>
<td></td>
<td>Prolongation of phospholipid-dependent coagulation test</td>
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<td></td>
<td>Evidence of an inhibitor shown by mixing studies</td>
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<tr>
<td></td>
<td>Confirmation of the phospholipid-dependent nature of the inhibitor</td>
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<tr>
<td>Screening</td>
<td>Dilute Russell viper venom time (DRVVT)</td>
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<td></td>
<td>Kaolin cephalin clotting time (KCT)</td>
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<td></td>
<td>TTI</td>
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<tr>
<td>Tests for anticardiolipin antibodies</td>
<td>Solid-phase ELISA immunobassey for IgM, IgG, IgA</td>
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<tr>
<td></td>
<td>β-2-glycoprotein I (β2-GPI)</td>
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<tr>
<td></td>
<td>ELISA for detection of other negatively charged phospholipids</td>
</tr>
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ELISA is enzyme-linked immunosorbent assay.
dependent coagulation reactions, thus prolonging coagulation times [9]. The term “lupus anticoagulant” is a misnomer, since this phenomenon is not seen exclusively in SLE patients. A number of methods have been used to detect the presence of lupus anticoagulant, including modifications of the activated partial thromboplastin times (aPTT), the kaolin cephalin clotting time (KCT), the dilute Russell viper venom time (dRVVT), and the thromboplastin inhibition test (TTI). The dRVVT is considered to be one of the most sensitive tests for lupus anticoagulant.

Tests for anticyclophilipid antibodies
Most patients with APS are identified with elevated anticyclophilipid antibodies. The precursor for this assay, the biologic false positive test for syphilis developed at the Venereal Disease Research Laboratory, is itself an anticyclophilipid test in which cardiolipin is the antigen. High levels of anticyclophilipid antibodies are predictive of an increased risk of thrombosis [8]. It should be emphasized that positive anticyclophilipid antibodies assays may be found in the asymptomatic “normal” population with a prevalence that varies from 3 to 10% [8]. It should also be appreciated that in some cases of APA positivity in patients with renal disease, such as in acute post-streptococcal glomerulonephritis, the presence of APAs may be a marginal immunological phenomenon triggered by streptococcal infection, unrelated to the glomerular disease [115]. Other anticyclophilipid tests include antibodies against β2-GP1, the major protein cofactor for APAs, and antibodies against phosphatidyl ethanolamine. Enzyme-linked immunosorbent assays (ELISAs) for β2-GP1 antibodies are considered to be more specific, but less sensitive for APS than anticyclophilipid assays [111]. Clinical correlation is therefore necessary to decide the significance of a positive APA test. This comprehensive diagnostic approach (Table 3) often includes using a combination of coagulation-based tests for lupus anticoagulant and solid-phase ELISAs for anticyclophilipid antibodies. Such an approach would help resolve the “equivocal” cases of APS [116, 117].

Treatment
Initiation of anticoagulation is indicated in thrombosis associated with APS [118–120]. This should be considered the mainstay of therapy in this syndrome. The goal is to maintain an international normalized ratio (INR) of 3.0 or higher. Anticoagulation therapy is sometimes supplemented with aspirin, although aspirin alone is less effective. The duration of anticoagulation is unknown, but lifelong anticoagulation is favored, as the risk recurrence is very high. Premature cessation of anticoagulation may also precipitate CAPS [61]. Preliminary data from Ruiz-Irastorza et al in a cohort of patients meeting the strict criteria for APS [5] shows that a target INR of 3.5 offers good protection against recurrences without a major increase in hemorrhages. (abstract; Ruiz-Irastorza G et al, Arthritis Rheum 43:S313, 2000). The factors that trigger recurrent thrombosis are poorly understood [120]. While steroids may decrease APA titers, they do not reduce the risk of thrombosis. Recently, it has been shown that steroids may also potentiate the anticoagulant effect of warfarin [121]. Scattered case reports suggest that plasmapheresis is beneficial in APS patients presenting with TMA [122, 123]. It should also definitely be considered in the patients presenting with CAPS, as it removes the pathogenic APAs, inducing the widespread thrombotic diathesis. The role of thrombolytics is unclear at this time, although thrombolytic therapy improved outcome in one patient with acute large-vessel occlusive disease [124]. The role of hydroxychloroquine and/or chloroquine in preventing thrombosis in APS is controversial. Some data suggest that these drugs have antithrombotic actions. Platelet aggregation induced by adenosine diphosphate (ADP), collagen, and ristocetin can be inhibited by chloroquine in a dose-dependent manner [125–127]. Consistent with these observations, hydroxychloroquine significantly diminished both thrombus size and total time of thrombus formation in mice with APS [128]. However, the effect of chloroquine in APS in humans is unclear. Experience with intravenous immunoglobulins in treating APS is limited and uncontrolled. Some data suggest that it is effective for the prevention of recurrent pregnancy loss when therapies such as heparin and low-dose aspirin have failed [128-131]. Its role in treating renal disease in APS is unknown at this time, although intravenous immunoglobulin in combination with steroids and heparin improves survival in CAPS. Recently, we showed that immunoglobulin decreases in vivo thrombosis and endothelial cell activation in animal models of APS [131, 132]. Newer therapeutic modalities that deserve further investigation include prosta-cyclic, fibrinolytic agents such as tissue plasminogen activator (TPA) and defibriotid. Some investigators have shown that tolerance can be induced to experimental APS by syngeneic stem cell transplantation [132].

ACKNOWLEDGMENTS
We thank Sylvia Wadley for secretarial assistance. Dr Pierangeli is supported by an NIH grant # NIGMS GM082248. We thank Donald Wesson, M.D., for critical manuscript review and Pat Abramson of the division of Information Graphics at Morehouse for his help with Figure 1.

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