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D.R. Wagenknecht
D.R. Fastenau
R.J. Torry
D.G. Becker
W.M. LeFor
C.B. Carter
B.W. Haag
J.A. McIntyre

# Risk of early renal allograft failure is increased for patients with antiphospholipid antibodies

D.R. Wagenknecht · D.R. Fastenau · R.J. Torry · C.B. Carter · B.W. Haag · J.A. McIntyre
Renal Transplantation Program, Methodist Hospital of Indiana, 1701 N. Senate Boulevard, Indianapolis, IN 46202, USA

J.A. McIntyre (✉)
Transplantation Immunology Laboratory, Methodist Hospital of Indiana, 1701 N. Senate Boulevard, W116, Indianapolis, IN 46202, USA

R.J. Torry
College of Pharmacy and Health Sciences, Drake University, 2507 University Avenue, Des Moines, IA 50311, USA

D.G. Becker · W.M. LeFor
Transplantation Immunology Laboratory, LifeLink Foundation, 409 Bayshore Boulevard,Tampa, FL 33606, USA

**Abstract** Renal allograft thrombosis can cause transplant failure. Because antiphospholipid antibodies (aPA) are associated with thrombosis, we investigated pretransplant sera from patients with early renal allograft failure to determine if aPA were present. Fifty-six final crossmatch (FxM) sera from patients whose transplant failed within 16 days were compared to FxM sera from the next sequential transplant patients. The sera were tested for IgG, IgM, and IgA antibodies to cardiolipin, phosphatidylserine, and phosphatidylethanolamine. aPA were identified in 57% of FxM sera from patients with early non-function versus 35% of FxM sera from patients with functioning grafts ($P = 0.02$). Historical sera from 11 aPA-positive patients contained aPA up to 18 months prior to transplantation. Since aPA were present in historical sera, testing for aPA can identify certain patients at risk for early allograft failure. The involvement of aPA in early allograft loss is supported by studies demonstrating aPA recovery from an explanted failed transplant.

**Key words** Antiphosphatidylserine antibodies · Antiphosphatidylethanolamine antibodies · Anticardiolipin antibodies · Thrombosis · Rejection

## Introduction

Colman and colleagues published the first report of immediate renal allograft thrombosis in 1969 [1]. Thrombosis limited to the donor kidney, in the absence of systemic coagulopathy, was considered a result of acute rejection or immunologically induced coagulopathy [1, 7, 10]. Despite advances in immunoregulatory drugs and increasingly sensitive laboratory tests to detect preformed anti-donor antibodies, transplants continue to be lost in the first days post transplantation. As recently as 1997, thrombosis occurring as late as the 2nd week posttransplant accounted for failure of 12.2% of primary transplants and 19.2% of repeat transplants in pediatric patients [9].

Antiphospholipid antibodies (aPA) are autoantibodies associated with venous and arterial thrombosis [14]. aPA detected by ELISA may bind the phospholipid (PL) antigen directly or, alternatively, may bind to plasma proteins which form complexes with the PL. Some of the PL-binding proteins are coagulation proteins (prothrombin, protein S, protein C, and high molecular weight kininogen) [6, 11, 12, 4, 16]. Other PL-binding plasma proteins, $\beta_2$-glycoprotein I and annexin V, bind to PL on the platelet and endothelial cell surfaces where they can sterically hinder formation of prothrombinase and tenase complexes. [4, 8]. Binding of aPA to these proteins may inhibit their natural anticoagulant effects. Furthermore, aPA bound to PL-binding plasma proteins adhered to endothelial cell membranes

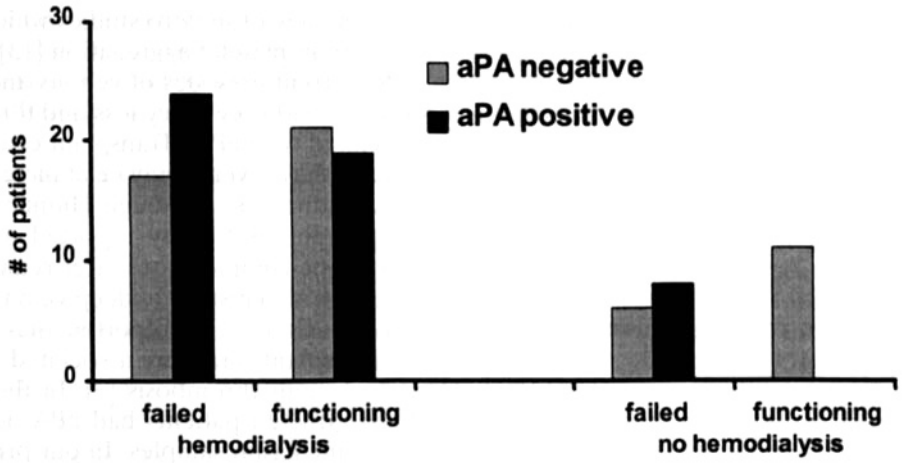


**Fig. 1** The effect of antiphospholipid antibodies (aPA) on transplant outcome for patients with and without prior maintenance hemodialysis. aPA-negative patients are represented by *gray bars* and aPA-positive patients by *black bars*. Study patients were grouped based on history of maintenance hemodialysis. Patients who received hemodialysis at any time prior to transplant are depicted on the *left side* of the graph, with chronic ambulatory peritoneal dialysis patients and patients transplanted without dialysis on the *right*. For hemodialysis patients, aPA did not predict transplant outcome ($P = 0.3766$). In contrast, aPA was associated with early transplant failure for all aPA-positive patients without exposure to hemodialysis ($P = 0.0022$)

cause endothelial cell activation in vitro [2]. These observations suggest that aPA-bound PL-binding proteins on the endothelial cell surface can stimulate a procoagulant condition.

In a case-controlled study of early renal allograft failure, we reported aPA present in 57% of 56 final crossmatch (FxM) sera from patients with early allograft failure compared to 35% of patients with functioning allografts ($P = 0.02$) [17]. We have further demonstrated the elution of aPA from a thrombosed renal allograft removed from a patient who had circulating aPA prior to transplant [18]. Immunohistochemical studies of biopsy material from this transplant nephrectomy demonstrated fibrin deposition in the renal vasculature. These data suggest that aPA may initiate thrombosis and promote fibrin deposition in the transplanted organ, which results in transplant failure.

## Materials and methods

### Patients

This retrospective study investigated FxM sera from patients transplanted at Methodist Hospital, Indianapolis, Ind., and LifeLink Foundation, Tampa, Fla., USA. FxM sera from 56 patients who experienced renal allograft failure in the first 16 days posttransplantation were investigated for the presence of aPA. For each transplant failure, sera from the next patient to receive a renal transplant at the same hospital was tested as a matched control. In two instances the next transplant also failed within the first 16 days, therefore, there were 54 controls with functioning allografts. When possible, longitudinal studies were performed with stored historical sera collected for monthly panel reactive antibody (PRA) screening while the patients awaited transplant. Detailed patient demographics have been described previously [17]. There were no significant differences in diagnosis for renal failure, or in type or duration of dialysis, between patients with early allograft failure and those with successful transplants.

### Antiphospholipid antibody ELISA

Serum samples were tested both in the presence and absence of exogenous PL-binding plasma proteins for IgG, IgA, and IgM anticardiolipin (aCL), antiphosphatidylserine (aPS), and antiphosphatidylethanolamine (aPE) antibodies by ELISA as described in detail elsewhere [17] Sera from 252 non-transplanted individuals were used to establish normal values for each of the aPA assays.

### Statistical analyses

Significance was determined by the two-tailed Fisher's exact test. A $P$ value equal to or less than 0.05 was considered significant.

## Results

When compared for aPA incidence, patients with a positive aPA value in any one of eighteen ELISA results was scored as positive. Patients with values within normal limits for all antibody isotype and PL antigen combinations were recorded as negative. Of 56 renal allograft failure patients, 32 (57%) tested positive for aPA at the time of transplantation compared to 19 (35%) of 54 patients with functioning allografts ($P = 0.0234$). Although IgG aCL was the most prevalent aPA specificity, many aPA-positive patients had multiple antibody specificities and isotypes. Historical sera from 11 primary allograft failure patients with

aPA-positive FxM sera were positive for the aPA specificity and isotype observed in the index sample. These longitudinal studies showed that aPA were present in patient sera as long as 18 months prior to transplantation.

When aPA findings were compared to patient demographics, no associations with cause of renal failure, diabetes, or other autoimmune diseases was apparent. There was no correlation with aPA and the development of PRA (current $P = 0.5767$; peak $P = 0.3559$). Patients who received a prior transplant(s) were not at increased risk for having aPA.

OKT3 induction therapy has been associated with renal allograft thrombosis, however, there were no differences in OKT3 induction therapy between aPA positive and -negative or functioning and failed allograft patients ($P = 0.4978$ and 0.6577, respectively). Elevated cyclosporine levels ( > 500 ng/dl) were observed more frequently for patients with functioning allografts than for patients with failed allografts. These data support the idea that aPA is a factor separate from immunosuppressive regimes that may contribute to allograft thrombosis.

Hemodialysis patients had a significantly higher incidence of aPA compared to patients who did not receive hemodialysis (43/81 vs 8/28; $P = 0.0293$). The method of dialysis was not known for one patient. No particular aPA specificity or isotype had predictive value for allograft loss in hemodialysis patients. In sharp contrast, aPA are a harbinger of transplant disaster in patients who never received hemodialysis. As shown in Fig. 1, all aPA-positive, non-hemodialysis patients experienced early renal allograft failure. These data suggest that aPA-positive renal allograft candidates who do not receive hemodialysis are at grave risk for early allograft failure.

## Discussion

We report that a significantly increased number of patients with primary renal allograft failure had aPA at the time of transplantation. A direct association of aPA with renal allograft loss was found in our previous study where we isolated aPA from an explanted, thrombosed renal allograft removed from an aPA-positive renal transplant patient [17]. The kidney, which functioned for only 4 h, was explanted on day 3 after ultrasound revealed no blood flow to the allograft. Biopsy findings from this kidney documented abundant fibrin deposition in the microvasculature. Retrospective analyses of the final donor crossmatch sera from this patient detected IgG aPE, aCL and IgA aPE antibodies, however, only IgG aPE antibodies were recovered from the failed kidney transplant. These observations suggest that aPE may be associated with renal allograft thrombosis, especially in view of in vitro studies which showed IgG aPE to augment platelet aggregation [13].

Recurrent episodes of venous and arterial thrombosis, recurrent pregnancy loss and thrombocytopenia are associated with aPA. Transplant candidates with one or more of these events may be at increased risk for having these antibodies. Although Humar and colleagues did not test for aPA, they reported a predisposition for thrombotic complications after renal transplantation in patients with a history of deep vein thrombosis [5]. Similarly, Ducloux et al. reported that aCL antibodies in posttransplant sera were associated with posttransplantation venous thrombosis [3]. In the latter study, 85% of the afflicted patients had aPA activity in their pretransplant serum samples. In our present study, historical serum samples were positive for the same aPA specificity and isotype observed in the FxM serum. Thus it would appear that screening patients for aPA prior to transplant would benefit certain patients by identifying those at risk for early renal allograft failure.

For transplant candidates with no hemodialysis exposure, the presence of aPA resulted in certain allograft failure ($P = 0.0022$). In contrast, aPA in patients receiving hemodialysis did not predict transplant outcome. One obvious difference between these patients is that hemodialysis requires heparin. Treatment for aPA-positive patients with thrombotic episodes has included heparin [14] We postulate that residual heparin from dialysis immediately pre-transplant may be protective for aPA-positive patients. Support for this possibility comes from in vitro studies which show that heparin can inhibit aPA binding and that certain aPA with anti-heparin specificity are neutralized by exogenous heparin [15]. Since transplant patients begin immunosuppresive therapy at the time of transplantation, the addition of heparin therapy may benefit aPA-positive patients.

Currently, all renal transplant candidates at Methodist Hospital are screened for aPA at the time they are listed for a cadaver donor transplant or prior to living-related donor surgery. Twenty-five percent of our patients listed in the United Network for Organ Sharing registry are aPA positive. Fractionated heparin (subcutaneous) has been prescribed for all aPA-positive patients immediately posttransplantation. To date, 15 aPA-positive patients have been treated with 30 mg fractionated heparin daily until their serum aPA values became negative. Although a few rejection episodes were documented, all 15 patients continue to experience successful outcomes (mean 327 days, range 21–800 days). Importantly, two patients were on chronic ambulatory peritoneal dialysis prior to transplantation. These patients continue to experience good renal function at 306 and 315 days post transplantation. This observation suggests that the adverse affects of aPA for patients who have never received hemodialysis may be overcome by posttransplantation heparin therapy.

The psychological and economic impact of early renal transplant failure are substantial. Because aPA are present in historical serum samples, screening for aPA can predict which patients are at increased risk for early renal allograft failure. Once identified, the aPA-positive patients may benefit from prompt heparin therapy subsequent to transplantation.

## References


1. Colman RW, Braun WE, Busch G, Dammin GJ, Merrill JP (1969) Coagulation studies in the hyperacute and other forms of renal allograft rejection. N Engl J Med 281: 685–691
2. Del Papa N, Sheng YH, Raschi E, Kandiah DA, Tincani A, Khamashta MA, Atsumi T, Hughes GR, Ichikawa K, Koike T, Balestrieri G, Krillis SA, Meroni PL (1998) Human beta 2-glycoprotein I binds to endothelial cells through a cluster of lysine residues that are critical for anionic phospholipid binding and offers epitopes for anti-beta 2-glycoprotein I antibodies. J Immunol 160: 5572–5578
3. Ducloux D, Pellet E, Fournier V, Rebibou JM, Bresson-Vautrin C, Rracadot E, Fellmann D, Chalopin JM (1999) Prevalence and clinical significance of antiphospholipid antibodies in renal transplant recipients. Transplantation 67: 90–93
4. Heerde WL van, deGroot PG, Reutelingsperger CPM (1995) The complexity of the phospholipid binding protein annexin V. Thromb Haemost 73: 172–179
5. Humar A, Johnson EM, Gillingham KJ, Sutherland DE, Payne WD, Dunn DL, Wrenshall LE, Najarian JS, Gruessner RW, Matas AJ (1998) Venous thromboembolic complications after kidney and kidney–pancreas transplantation. Transplantation 65: 229–234
6. Oosting JD, Derksen RHWM, Bobbink IWG, Hackeng TM, Bouma BN, deGroot PG (1993) Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanisms? Blood 81: 2618–2625
7. Rodriguez-Erdmann F, Guttmann RD (969) Coagulation in renal-allograft rejection. N Engl J Med 281: 1428
8. Schousboe I (1985) $\beta_2$-glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. Blood 66: 1086–1091
9. Singh A, Stablein D, Tejani A (1997) Risk factors for vascular thrombosis in pediatric renal transplantation. Transplantation 63: 1263–1267
10. Starzl TE, Boehmig HJ, Amemiya H, Wilson CB, Dixon FJ, Giles GR, Simpson KM, Halgrimson CG (1970) Clotting changes, including disseminated intravascular coagulation, during rapid renal-homograft rejection. N Engl J Med 283: 283–390
11. Sugi T, McIntyre JA (1995) Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen–PE complex. Blood 86: 3083–3089
12. Sugi T, McIntyre JA (1995) Plasma proteins required for antiphospholipid antibody detection. Nouv Rev Fr Hematol 37:S49–S52
13. Sugi T, McIntyre JA (1996) Autoantibodies to kininogen–phosphatidylethanolamine complexes augment thrombin-induced platelet aggregation. Thromb Res 84: 97–109
14. Triplett DA (1995) Antiphospholipid-protein antibodies: laboratory detection and clinical relevance. Thromb Res 78: 1–31
15. Wagenknecht DR, McIntyre JA (1992) Interaction of heparin with $\beta_2$-glycoprotein I and antiphospholipid antibodies *in vitro*. Thromb Res 68: 495–500
16. Wagenknecht DR, McIntyre JA (1994) Prothrombin: a factor in the antiphospholipid antibody (aPA) ELISA. Lupus 3: 364
17. Wagenknecht DR, Becker DG, LeFor WM, McIntyre JA (1999) Antiphospholipid antibodies are a risk factor for early renal allograft failure. Transplantation 68 (in press)
18. Wagenknecht DR, Fastenau DR, Torry RJ, Carter CB, Haag BW, McIntyre JA (1999) Antiphospholipid antibodies are a risk factor for early renal allograft failure: isolation of antiphospholipid antibodies from a thrombosed renal allograft. Transplant Proc 31: 285–288