Original Article

Are antiphospholipid antibodies clinically relevant in dialysis patients?

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Abstract. A cohort of 60 stable dialysis patients (56 haemodialysis, 4 continuous ambulatory peritoneal dialysis) was followed for 1 year to determine the relationship between anticardiolipin (aCL) antibodies and lupus anticoagulant (LA), and clinical events. The outcome measures were death, arterial, venous, and fistula thromboses, and fistula repairs. At baseline 15% had antibodies (5/60 IgG aCL, 3/60 LA, and 1/60 had both); 15 patients had a history of arterial thrombosis (1 patient was aCL positive), seven venous thrombosis (1 patient LA positive), 10 fistula thromboses (1 aCL positive); and 21 had a history of fistula repairs (4 aCL positive). Renal diagnosis, age (66.78 versus 59.67 years) and duration of dialysis (38.11 versus 45.25 months) were similar in patients with and without aCL antibodies or LA. Only the sex ratio showed a female predominance in the aCL- or LA-positive patients compared to the negative patients (3M:6F versus 36M:15F), but this was not significant (P = 0.07). After 1 year there were 10 deaths (1 LA positive), 12 thrombotic events in eight patients (none aCL or LA positive), and 21 had a history of fistula repairs in seven patients (1 aCL-positive patient). We are unable to show higher rates of death, thrombotic event, or fistula repair in dialysis patients with aCL antibodies or LA followed up for 1 year in one centre. The clinical importance of antiphospholipid antibodies in dialysis patients is uncertain.

Key words: anticardiolipin antibodies; antiphospholipid antibodies; continuous ambulatory peritoneal dialysis; end-stage renal disease; haemodialysis; lupus anticoagulant

Introduction

Anticardiolipin antibodies (aCL) and the lupus anticoagulant (LA) are antiphospholipid (APL) antibodies that have been associated with arterial and venous thromboses, neurological diseases, thrombocytopenia, and fetal loss in patients with systemic lupus erythematosus (SLE) [1]. It was the development of an immunoassay to aCL antibodies that led to extensive research into their clinical role [2]. APL antibodies have been found in several non-SLE diseases, but are rarely found in a healthy population [3]. There is evidence that in non-SLE patients they are associated with a similar pattern of clinical manifestations as in SLE, 'the primary antiphospholipid syndrome' [4].

In end-stage renal disease (ESRD), Quereda et al. found LA in 30% of haemodialysis patients, compared to 11% of those receiving conservative treatment [5]. The incidence of thrombotic episodes was 23% in LA-positive patients and 13% in LA-negative patients, but this was not significant. Later, in Finland, 112 haemodialysis patients and 34 continuous ambulatory peritoneal dialysis (CAPD) patients were tested for aCL antibodies, and 23% were positive [6]. The authors suggested that the aCL-positive patients...
had an increased rate of thrombotic events. Garcia-Martin et al. tested both LA and aCL antibodies in 51 haemodialysis patients and found rates of 31% for aCL antibodies, 22% LA, and 37% for either LA or aCL [7]. Twenty-two conservatively treated ESRD patients had an aCL prevalence of 18%. The clinical relevance of these data is unknown because of their cross-sectional nature.

We present the results of a prospective clinical study in which a cohort of patients were followed up for 1 year, to determine the clinical relevance of aCL antibodies and LA in ESRD.

**Subjects and methods**

All stable dialysis patients (56 haemodialysis; 4 CAPD) in our unit (in a university/affiliated district general hospital) were studied from 15 May 1990 to 15 May 1991. The mean age was 61 years (range 29–84); 39 male, 21 female; mean duration of dialysis 44 months (range 3–286). Renal diagnoses were nephroangiosclerosis 19 patients (32%, 5 biopsy proven); analgesic nephropathy 12 (20%); chronic glomerulonephritis 10 (17%, 5 biopsied); polycystic kidney disease 4; diabetic nephropathy 4 (2 biopsied); obstrucive or reflux nephropathy 4; polycystic kidney disease 2 (both biopsied); primary amyloidosis 1 (biopsy proven); secondary amyloidosis 1 (biopsy proven); Wegener's granulomatosis 1 (biopsy proven); Henoch-Schönlein purpura 1 (biopsy proven); chronic pyelonephritis 1. In the five unbiopsied patients with presumed chronic glomerulonephritis, the diagnosis was based on proteinuria (more than 1.5 g/day), and the presence of bilateral small smooth kidneys on ultrasound. No patients had SLE, or satisfied the criteria for the 'primary antiphospholipid syndrome' [1], and none received chlorpromazine.

Baseline data were gathered on the artificial kidney type, history of arterial, venous, and fistula thromboses, and fistula repairs. During the monthly routine blood sampling, extra predialysis samples were drawn (through the fistula for 30 min, and stored at –20°C within 2 h), VDRL (10 ml serum, centrifuged and stored at –20°C within 2 h), aCL antibodies (10 ml serum, centrifuged and stored at –20°C within 2 h), VDRL (10 ml serum), and antinuclear factor (ANF, 10 ml serum). Platelet counts (Coulter counter), APTT (reagents from Diagostica St Louis, USA) 50 ug/ml, diluted in methanol was coated on microtitre wells. After post-coating with bovine serum albumin 1% in phosphate-buffered saline (PBS-BSA), serum 1 50 PBS-BSA was added. Anticardiolipin antibodies were detected using peroxidase-labelled F(ab)2 goat antihuman IgG (Cappel, Worthington, USA). Results were calibrated with international reference sera provided by the Antiphospholipid Standardization Laboratory (University of Louisville, KY, USA) and expressed in GPL units. Healthy controls had titres ranging between 3 and 6 GPL units, while positive control patients (mainly SLE) ranged between 2 and 200 GPL units. APTTs greater than 43 s, ANF more than 1.40, and aCL more than 10 GPL units were considered as abnormal. Laboratory colleagues were blind to clinical data.

The cohort was followed up for 12 months. The outcome measures were death; arterial, venous, and fistula thromboses; and the number of fistula repairs. Arterial thromboses (acute myocardial infarction, unstable angina, cerebral infarction, transient ischaemic attack, peripheral arterial occlusion) and venous thromboses (pulmonary, deep-venous, portal, or renal vein) were diagnosed using standard clinical criteria, and were confirmed by electrocardiograms, computed tomography, angiography, venography, or duplex scanning. Fistula thrombosis was always confirmed by fistulography.

Statistical analyses were performed using the chi-square test with Yates's correction for continuity or the unpaired t-test, with P < 0.05 taken as the level of significance.

**Results**

**Prevalence of aCL antibodies or LA**

Twelve patients had a prolonged APTT (20%), two of whom were LA positive. Nine patients had aCL antibodies or LA (15%). Six patients were positive for aCL antibodies (10%), one of whom was also positive for LA. The mean aCL antibody value was 22.83 GPL units (range 11–66), 5 of 6 patients had titres between 10 and 20 GPL units, and only one had titres greater than 20 GPL units. A total of four patients were LA positive, but only one of these had a LA as an isolated finding. Three patients had a platelet count lower than 100 x 10⁹/l, one of whom was also LA positive with a prolonged APTT, the second with a prolonged APTT, while in the third this was an isolated finding. One patient was ANF positive (1/320), also an isolated finding. There was no patient with a false positive VDRL.

**Past history**

Baseline clinical data are shown in Table 1. There was no association between a history of thrombosis and aCL or LA. The one LA-positive patient had a renal diagnosis of chronic pyelonephritis, a history of venous thrombosis and pulmonary hypertension (documented at right-heart catheterization), a platelet count of 84 x 10⁹/l, and a prolonged APTT. Neither
aCL nor LA had been previously measured, so although this patient probably had the primary antiphospholipid syndrome; this was unknown during the study.

The number of aCL- or LA-positive patients with a history of fistula repairs was not higher than expected (DF = 1, P = 0.93). There were proportionally more females in the aCL- or LA-positive group (male:female ratio 3:6), compared to the negative patients (35:15), but this was not significant (P = 0.07; chi square with Yates's correction). Renal diagnosis, age (66.78 versus 59.67 years; P = 0.13), and duration of dialysis (38.11 versus 45.25 months; P = 0.69) were similar in patients with and without aCL antibodies or LA (two-tailed unpaired t test).

### Dialysis details

Renal diagnoses in the aCL-positive patients were chronic glomerulonephritis 2 (1 biopsied), diabetic glomerulopathy 1 (biopsied), obstructive nephropathy 1 (no biopsy), and nephroangiosclerosis 1 (no biopsy). In LA-positive patients the renal diagnoses were analgesic nephropathy 1 (no biopsy), chronic pyelonephritis 1 (no biopsy), and polyarteritis nodosa 1 (biopsied). The patient positive for both aCL and LA had analgesic nephropathy (no biopsy). These data are difficult to evaluate due to the small number of aCL- or LA-positive patients, however 4 of 9 had either chronic glomerulonephritis or chronic interstitial nephritis due to analgesic abuse, and this was similar to the general dialysis population. Two of seven cuprophane dialysed patients had aCL or LA compared to 7 of 53 treated with more biocompatible membranes or CAPD (Table 2), this was not statistically significant (DF = 1, P = 0.73).

### Events during follow-up

After 1 year of follow-up (Table 3), there were 10 deaths (17%), 12 thrombotic events in eight patients (5 patients had a single thrombotic event, while 3 had multiple events), and nine fistula repairs in seven patients (5 patients had one repair, while 2 needed multiple repairs). No patient with aCL or LA had a thrombotic event during follow-up. Only one of seven patients requiring a fistula repair was aCL positive (66 GPL units; LA-negative, platelets and APTT normal), but the repair was done for severe bleeding from an infected fistula, and not thrombosis. In all there were 16 patients with events (26.7%), while 39 remained stable and five were transplanted but remained stable. Of the 44 stable, event-free patients, seven had APL antibodies (16%), including the only patient positive for both LA and aCL (11 GPL units). This patient had no history of thrombosis or fistula repair in 84 months of dialysis.

### Deaths

The non-thrombotic causes of death were infection in two patients, carcinoma in two, heart failure (one idiopathic cardiomyopathy, and one due to primary amyloidosis), withdrawal of dialysis in one, and subdural bleeding in one. Two patients died of thrombosis; one, who died with multiple cerebral infarctions, has been described [10], and was negative for aCL and LA. The second patient died suddenly at home (no autopsy performed, presumed sudden cardiac death), but was aCL and LA negative.

The only death in a LA-positive patient was due to infection (pulmonary sepsis) and adult respiratory distress syndrome in a 68-year-old female with biopsy-proven polyarteritis nodosa. There was no evidence of thrombosis in this patient, despite her having LA and a prolonged APTT. No patient positive for aCL antibodies died.


Discussion

This is the first prospective clinical study of aCL antibodies and LA in dialysis patients. Our conclusions must be tempered by the small number of patients found to be positive for these antibodies (a prevalence of 15% compared to the 22–37% reported by previous cross-sectional studies [5–7]). This makes our study vulnerable to type 2 errors. In addition, five of our six aCL-positive patients had low titres (between 10 and 20 GPL units), and only one had a titre greater than 20 GPL units. The low numbers of positive patients and the low titres may explain why we failed to detect a difference in clinical outcome. Further, the study was not designed to assess the laboratory evolution of aCL and LA during follow-up. In particular, we do not know if patients positive for aCL or LA at baseline remained so during the year. Nor do we know whether initially negative patients developed aCL or LA at the time of thrombotic complications.

The mechanisms of APL antibody production and thrombogenesis are unknown. In dialysis patients Garcia-Martin et al. speculated that a combination of immune dysfunction, due to uraemia, and dialysis membrane incompatibility led to APL production [7]. We used far fewer cuprophane membranes than previous series, and this may explain our lower prevalence of APL antibodies compared to Garcia-Martin et al. (15% versus 37%). Quereda et al. used only cuprophane membranes and found a LA prevalence of 30% [5]. We found a 29% (2 of 9) prevalence of APL antibodies in our cuprophane-treated patients, compared to 15% (7 of 46) in those treated with biocompatible membranes. There was no significant difference in APL prevalence in cuprophane-dialysed patients compared to those treated with more biocompatible membranes, in our series. However, our numbers are too small to test the hypothesis that cuprophane membranes lead to a greater production of APL antibodies.

Are there other factors which may account for our lower prevalence and titres of aCL and LA? Our patients had been on dialysis for similar lengths of time (44 months) as those of the other published series (37–52 months). Like us, Quereda et al. [5] and Garcia-Martin et al. [7] had no SLE patients, while Grönhagen-Riska et al. had only 6/146 patients with SLE [6]. There was a female preponderance in our group of APL-positive patients (67%), compared to the APL-negative patients (31%), which was not significant (P = 0.07). In our unit as a whole there was a male preponderance (65%), and it is possible that the lower prevalence of APL antibodies was due to the small number of female patients (and a type 2 error). However, Garcia-Martin et al. had more females in general (29 of 51), but fewer in the aCL group (9 of 20), thus opposite to our findings. Quereda et al. had sex ratios similar to our cohort, but found no relation between gender and LA. Elderly females may develop autoantibodies (such as ANF and rheumatoid factor) more frequently, but a recent survey of autoantibodies in 100 (38 female) healthy elderly British found no subject with raised aCL antibodies [11]. Thus there is little evidence that gender plays a role in APL genesis in dialysis patients.

The low prevalence and level of aCL and LA in this series may be due to differences in age and renal diagnosis. Our patients were older (mean age 61 years) than those of other studies (42–53 years), and the prevalence of chronic glomerulonephritis (17%) was less (Quereda et al., 35% [5]; Grönhagen-Riska et al., 30% [6]). A higher prevalence of aCL antibodies has been found in patients with chronic glomerulonephritis [6].

Another feature of our study was the apparent dissociation of LA and aCL results (only one patient had both APL antibodies). This is in contrast to overlap rates of more than 60% in selected populations [4,12]. This could have been due to technical factors such as sample storage times or laboratory methods. However, both tests were performed in experienced laboratories [8,13], and aCL antibodies were measured in comparison with internationally accepted standards [9]. Further, Garcia-Martin et al. also found a dissociation between aCL and LA in dialysis patients [7], and others have also noted a lack of concordance between the two tests not accounted for by differences in test sensitivity [1]. This study was not designed to explain the discordance between aCL and LA, but we suspect that this may be an effect of haemodialysis.

Despite finding only 9 of 60 patients positive for aCL antibodies or LA, we were able to ensure close prospective clinical study over 1 year. The strength of this study is that there was no episode of thrombosis in a positive patient, while there were 12 thrombotic events recorded in eight negative patients during the year. The only patient positive for LA or aCL antibodies that died did so because of sepsis, not thrombosis. Additionally, of nine fistula repairs in seven patients, only one repair was necessary in an aCL-positive patient, and this was not because of thrombosis. We diagnosed the primary antiphospholipid syndrome in one patient, after receiving the positive LA result, and she remained stable during the follow-up period.

This is the first prospective clinical study of APL antibodies in dialysis patients. We have been unable to show an increased rate of death, thrombotic event, or fistula repair in nine patients with aCL antibodies or LA followed up for 1 year in one centre. There
appears to be no association between dialysis technique, length of dialysis or past history of thrombosis with these antibodies. The clinical relevance of the raised prevalence of aCL antibodies and LA in dialysis patients without SLE is uncertain. Indirectly this study supports the suggestion of Garcia-Martín et al. that haemodialysis-related APL antibodies are clinically silent, and are isolated biochemical abnormalities. Further cross-sectional studies are unnecessary. However, larger prospective studies may help to define the influence of renal diagnosis and membrane type on APL antibody genesis, the evolution of antibody levels, and the clinical importance of these antibodies in dialysis patients.

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References


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