Anticardiolipin Antibodies Predict Early Recurrence of Thromboembolism and Death among Patients with Venous Thromboembolism following Anticoagulant Therapy

Sam Schulman, MD, Elisabet Svenungsson, MD, Staffan Granqvist, MD, and the Duration of Anticoagulation Study Group*

PURPOSE: To compare the risk of recurrent venous thromboembolism in patients with and without antiphospholipid antibodies.

PATIENTS AND METHODS: Anticardiolipin antibodies were tested 6 months after a first or second episode of venous thromboembolism. Of the patients with a first episode of venous thromboembolism only the 412 who received 6 months of anticoagulation were studied. Two hundred and eleven patients with a second episode received oral anticoagulation for 6 months or indefinitely. The therapy was targeted at an international normalized ratio (INR) of 2.0 to 2.85. All patients were followed up for 4 years after enrollment.

RESULTS: Among the 412 patients with a first episode of venous thromboembolism the risk of recurrence was 29% in patients with anticardiolipin antibodies and 14% in those without antibodies ($P = 0.0013$). In those with antibodies, there was an increased risk during the first 6 months after cessation of anticoagulation. The risk of recurrence increased with the titer of the antibodies. Four-year mortality rate was 15% in those with antibodies and 6% in those without ($P = 0.01$). Among 34 patients with a second event of venous thromboembolism and anticardiolipin antibodies, there were no recurrences during anticoagulant therapy versus 20% in those who received only 6 months of treatment ($P = 0.08$).


Antiphospholipid antibodies are directed toward negatively charged phospholipids or proteins associated with phospholipids, such as $\beta_2$-glycoprotein 1. Patients with these antibodies who have recurrent venous or arterial thrombosis, fetal loss, or thrombocytopenia are said to have the antiphospholipid syndrome. In the absence of concurrent systemic lupus erythematosus, the condition is called the primary antiphospholipid syndrome (1).

The etiology of these antibodies is not known. Furthermore, questions remain regarding the critical titer and persistence of the antibodies, as well as the optimal type, intensity, and duration of secondary preventive therapy (2).

Elevated titers of anticardiolipin antibodies may be present before the first occurrence of venous thromboembolism (3). The presence of antiphospholipid antibodies is associated with a higher risk of thrombotic recurrence, and prolonged oral anticoagulation is recommended (4–9). Most of those studies included patients with venous and arterial thrombotic disease as well as primary antiphospholipid syndrome or that associated with systemic lupus erythematosus. The risk of recurrent venous thromboembolism may be high after discontinuation of oral anticoagulation in patients with systemic lupus erythematosus (9,10). There have been three studies of patients with venous thromboembolism who did not have systemic lupus erythematosus (11–13). In two of the studies the presence of anticardiolipin antibodies conferred an increased risk of recurrence (11,12), whereas the third study found that lupus anticoagulant, but not anticardiolipin antibodies, was associated with recurrence (13). All three studies had relatively few cases with lupus anticoagulant or anticardiolipin antibodies, and the durations of anticoagulation and follow-up were not uniform.
In two trials, comparing different durations of oral anticoagulation after a first and second event of venous thromboembolism, we analyzed anticardiolipin antibodies after 6 months. All patients were followed up for 4 years. We have assessed the data with respect to risk of recurrence, mortality, and protective effect of the secondary prophylaxis.

**PATIENTS AND METHODS**

*Patients*

Patients were recruited during 3 years for a multicenter trial at 16 hospitals in Sweden of the optimum duration of oral anticoagulation after venous thromboembolism. A total of 1,124 patients of at least 15 years of age were included, 897 with a first episode of deep vein thrombosis or pulmonary embolism (14) and 227 patients with a second episode (15). Main exclusion criteria were previously diagnosed malignant disease (152), severe current disease (137), insufficient ascertainment of venous thromboembolism (80), unwillingness to participate (80), alcohol abuse (22), previous venous ulcer (8), and congenital deficiency of an inhibitor of coagulation (antithrombin, protein C or S—only tested in patients under 50 years of age or with a positive family history) (5).

*Diagnostic Criteria*

The initial diagnosis was objectively confirmed in all cases (14,15). Ascending phlebography was the sole method used for the diagnosis of deep-vein thrombosis, whereas pulmonary embolism was verified with angiography or the combination of chest radiography and radioactive isotope perfusion and ventilation lung scanning. Recurrent deep-vein thrombosis was also diagnosed with phlebography, showing deep-vein thrombosis in another extremity or in another deep vein of the same leg as the original thrombus or deep-vein thrombosis in the originally affected veins with either a new proximal extension of at least 5 cm or, if proximal limit of the original thrombus had not been visualized, the presence of a constant filling defect, surrounded by contrast. Recurrent pulmonary embolism was verified by lung scanning, showing defects in originally perfused areas, or at the initial location provided that another examination during the intermediate period had demonstrated normalized perfusion. Autopsy was required for verification of fatal embolism. A radiologist, blinded to the treatment, laboratory results, and dates of the examinations, reviewed all phleograms and lung scans from patients with recurrent events.

*Treatment*

The initial treatment of the qualifying thromboembolic event consisted in the majority of patients of unfractionated heparin, administered intravenously or subcutaneously for at least 5 days until a prothrombin time within the target range was reached. Forty-seven patients were treated with low-molecular-weight heparin instead, and 30 patients received thrombolytic therapy before heparinization. Oral anticoagulation with warfarin sodium or dicoumarol was targeted to an international normalized ratio (INR) of 2.0 to 2.85, using Stago Prothrombin-complex Assay (Diagnostic Stago, Paris, France) or Nycotest PT (Nycomed, Oslo, Norway) as the thromboplastin reagents. During maintenance treatment, thrombokin time tests were repeated weekly for the first month and then at least every 4 weeks. A quality control program was used to ensure treatment of comparable intensity at the different centers (16). Graded compression stockings were prescribed to all patients with deep-vein thrombosis.

*Study Design*

Randomization, which was performed centrally, took place at the end of the hospitalization, before the first test for anticardiolipin antibodies. Patients with a first thromboembolic event were randomly allocated to oral anticoagulation for 6 weeks or 6 months, and those with a second event for 6 months or indefinitely. The patients were informed (and repeatedly reminded) about the symptoms of deep-vein thrombosis and pulmonary embolism and told to report immediately to the emergency room of their center if any such symptoms occurred. End points were death, recurrent venous thromboembolism or hemorrhage necessitating hospitalization, infusion with blood products, or treatment with vitamin K. Patients lost to follow-up were repeatedly checked with the Death Registry and with the registry of hospitalizations; vital status was 100% complete.

Blood sampling for anticardiolipin antibodies was done at the 6-month follow-up after the qualifying thromboembolic event. Patients with abnormal results were tested after another 6 months and then yearly.

*Laboratory Method*

Serum was analyzed at four laboratories for the presence of immunoglobulin G anticardiolipin antibodies by enzyme-linked immunosorbent assay (ELISA), that incorporated rabbit antihuman IgG conjugant. Microtiter plates were coated with cardiolipin antigen (Sigma Chemical Company, St Louis, Missouri) and air dried at +4°C overnight. The plates were blocked with 1% bovine serum albumin or 10% fetal calf serum in 100 µL phosphate-buffered saline at 4°C for 2 hours or overnight or at room temperature for 1 hour. The serum was diluted 1:60 in 1% bovine serum albumin in phosphate-buffered saline or 1:10 in 0.05% Tween in phosphate-buffered saline or 1:100 in 10% fetal calf serum, and incubated in the wells at room temperature for 1 hour, 3 hours, or 4 hours. The plates were washed, and the rabbit antihuman IgG conjugant, diluted 1:500 in 1% bovine serum albumin in phosphate-buffered saline or in 0.05% Tween in phos-
phosphate-buffered saline or in 10% fetal calf serum, was added to the well and incubated for 1 hour, 90 minutes, or overnight. The substrate, para-nitrophenylphosphate (Sigma Chemical Company) or orthophenylenediamine (Dakopatts), was added to the wells, and the plates were incubated for 30 minutes. The optical densities were measured at 405 nm or 490 nm. As reference, three of the laboratories used five sera of known titers or five freeze-dried test samples from Anti-Phospholipid Standardization Laboratory, University of Louisville, Louisville, Kentucky. One unit was the standard deviation of a large number of normal sera or at one laboratory reported as GPL-units, defined as the cardiolipin-binding activity of 1 µg/mL of an affinity purified IgG anticardiolipin preparation from a standard serum (REY). Complete details can be obtained from the authors.

At the beginning of this study the National Bacteriological Laboratory had distributed 15 different reference samples to these laboratories. No false positive results were observed, and strong positive results were also uniform. Since the method at one laboratory differed significantly from the others and used another type of units, all samples from that laboratory were also tested in parallel at another laboratory. The prevalence of positive anticardiolipin antibodies was 2% in a population based sample of normal material of adults.

**Statistical Analysis**

The titers of anticardiolipin antibodies were grouped according to the classification of the National Bacteriological Laboratory and the comparison between the different laboratories into normal range (<5 GPL units), weak positive (5 to 35), moderate positive (36 to 150), and strong positive (>150). Statistical analyses were performed on an intention-to-treat basis unless otherwise stated. All analyses are based on data from the first 4 years. The event-free survival was compared with log-rank test, chi-square test, or Fisher’s exact test for two or three groups. We estimated 95% confidence intervals (CI). All analyses were performed in SAS (Cary, North Carolina).

The study was approved by the regional and local ethics committees.

**RESULTS**

Patients with a first or second episode of venous thromboembolism were recruited during the period April 12, 1988 to April 18, 1991. Most patients had deep-vein thrombosis (Table 1). We were unable to obtain samples for anticardiolipin antibodies for between 5% and 9% of the patients due to death or severe, intercurrent disease before the 6-month follow-up.

Among patients with a first episode of venous thromboembolism, 10 patients received shorter, and 14 received longer, anticoagulation than intended, but this did not affect the mean duration of treatment. Nine patients with a second venous thromboembolism who had been randomized to the 6-month group received treatment for 7 to 48 months owing to additional indications during follow-up (atrial fibrillation, transitory ischemic attacks, artificial heart valve), so that the actual mean duration was 7.2 months. Twenty-two patients with a second event and allocation to indefinite anticoagulation had premature discontinuation due to side effects, poor compliance, or refusal to continue, reducing the actual mean duration during the first 4 years to 43.7 months.

The prevalences of titers of anticardiolipin antibodies above the normal range were similar in men (13.7%) and women (17.1%).

### Table 1. Characteristics of the Patients According to Episode of Venous Thromboembolism and Allocation to Duration of Anticoagulation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First Episode Treatment Allocation</th>
<th>Second Episode Treatment Allocation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 Weeks (n = 443)</td>
<td>6 Months (n = 454)</td>
</tr>
<tr>
<td>Tested for anticardiolipin antibodies, n (%)</td>
<td>398 (90)</td>
<td>412 (91)</td>
</tr>
<tr>
<td>Deep-vein thrombosis/pulmonary embolism, n</td>
<td>350/48</td>
<td>363/49</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>60.8</td>
<td>60.2</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>179 (45)</td>
<td>181 (44)</td>
</tr>
<tr>
<td>Antibody positive, n (%)</td>
<td>48 (12)</td>
<td>68 (17)</td>
</tr>
</tbody>
</table>

**Anticardiolipin Antibodies and Venous Thromboembolism/Schulman et al**

April 1998 THE AMERICAN JOURNAL OF MEDICINE® Volume 104
with anticardiolipin antibodies may have been missed in the 6-week group before blood sampling at 6 months. The analysis is therefore based only on the 412 patients with a first episode of thromboembolism who were anticoagulated for 6 months. During 4 years of follow-up, there were 67 recurrent venous thromboembolic events. The recurrence risk was 29% (20 of 68) in patients with anticardiolipin antibodies and 14% (47 of 334) in patients without antibodies \((P = 0.0013)\), for a risk ratio of 2.1 (95% CI 1.3 to 3.3; Table 2). The rates of recurrence were 0.10 per year in patients with anticardiolipin antibodies, and 0.04 per year in those without antibodies. The risk of recurrence was 28% (17 of 60) in patients with a low positive titer of anticardiolipin antibodies (5 to 35 GPL units), and 3 of 8 in patients with a moderate or high titer (>35 GPL units). Proximal deep vein thrombosis was more common in the antibody positive group than in the negative group (71% versus 54%, \(P = 0.02\)), which may have contributed to the increased risk of recurrence among the former.

The cumulative risk of recurrent venous thromboembolism is shown in Figure 1. The 2 patients who experienced recurrences before 6 months had stopped oral anticoagulation prematurely. Whereas there was a linearly increasing risk among the patients without anticardiolipin antibodies, those with positive titers had a sharp rise in recurrence risk after discontinuation of anticoagulation. After 1 year, risk seems to become linear in this group as well, but with a steeper slope, corresponding to 5.6% per year versus 3.7% in patients without anticardiolipin antibodies.

Of the 20 cases with anticardiolipin antibodies who developed recurrent events, the titer was still positive in 6 cases, whereas 14 had become seronegative at that point.

Patients with a first recurrent episode of venous thromboembolism were randomly assigned to stop or to continue oral anticoagulation indefinitely beyond the time point of 6 months, when the sample for anticardiolipin antibodies was obtained in 211 patients. During 4 years of follow-up, there were 20 second recurrences in 90 patients without antibodies (22%) and 3 second recurrences in 15 with antibodies (20%) in the 6-month group. In the group with indefinite anticoagulation, 2 of 87 patients without antibodies and 1 of 19 with antibodies experienced a second recurrence (Table 2). These differences were not statistically significant. However, all 3 patients with second recurrences in the “indefinite treatment” group had discontinued the oral anticoagulation 2 to 10 months prior to the new event. By actual treatment the rate of a second recurrent venous thromboembolism in patients with anticardiolipin antibodies treated for 6 months was 3 of 15 and among those treated indefinitely 0 of 18 \((P = 0.08\) by Fisher’s exact test).

**Table 2.** Recurrences of the Thromboembolism during 4 Years of Follow-Up According to Presence of Anticardiolipin Antibodies

<table>
<thead>
<tr>
<th></th>
<th>First Recurrence</th>
<th>Second Recurrence</th>
<th>Second Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Six Months of</td>
<td>Indefinite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anticoagulation</td>
<td>Anticoagulation</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Antibody positive</td>
<td>20/68 (29%)</td>
<td>3/15 (20%)</td>
<td>1/19 (5%)</td>
</tr>
<tr>
<td>Antibody negative</td>
<td>47/344 (14%)</td>
<td>20/90 (22%)</td>
<td>2/87 (2%)</td>
</tr>
</tbody>
</table>

**Figure 1.** Cumulative probability of recurrent venous thromboembolism in patients after a first episode, anticoagulated for 6 months, accounting for loss from follow-up, by anticardiolipin antibody (ACLA) status.

**Figure 2.** Mortality in patients with a first episode of venous thromboembolism, anticoagulated for 6 months, by anticardiolipin antibody (ACLA) status.
Death

The cumulative risk of death by anticardiolipin antibody status, in patients with a first episode of venous thromboembolism who received 6 months of oral anticoagulation, is shown in Figure 2. The mortality risk becomes significantly higher among the patients with antibodies by month 24, and after 4 years it is 15% (10 of 68) in those with antibodies and 6% (20 of 324) in those without antibodies ($P < 0.01$), with a risk ratio of 1.8 (95% CI 0.9 to 3.6). The duration of oral anticoagulation did not affect mortality.

Data on malignancies were available for all patients. The incidence of cancer during 4 years of follow-up was 7% (11 of 150) in patients with antibodies and 6% (55 of 871) in patients without antibodies. Causes of death are shown in Table 3. If all thromboembolic causes are combined (pulmonary embolism, mesenteric vein thrombosis, myocardial infarction, and thromboembolic stroke) the risk of such death was 7% (CI 3 to 11) in patients with antibodies and 2.2% (CI 1.2 to 3.1) in those without antibodies ($P = 0.002$). The expected incidence of deaths due to those causes during 4 years in an age- and gender-matched population, based on available data from the entire Swedish population, is approximately 3%. Deaths were not more common in the period immediately after cessation of anticoagulation.

DISCUSSION

As opposed to most previous studies addressing the significance of antiphospholipid antibodies, our study is based on a sample of patients presenting with venous thromboembolism. In 15% of the patients tested after 6 months, elevated titers of anticardiolipin antibodies were found, corresponding to the APL-T type 1 syndrome (17). Only two of these patients turned out to have systemic lupus erythematosus. Our analysis of antiphospholipid antibodies was limited to IgG anticardiolipin antibody, which has been reported as the most frequently positive test, at least in patients with systemic lupus erythematosus and thromboembolism (5). The lupus anticoagulant, which is another common abnormality in these patients, was not analyzed in this study. We obtained the first sample for anticardiolipin antibodies 6 months after the index event to avoid transient seropositive states, influenced by the acute phase. Suspected recurrent events that were refuted by a negative venogram or lung scan occurred in similar proportions (about 4%) of patients without and with anticardiolipin antibodies, indicating that there was no bias toward more intense investigation in the antibody positive group. The increased prevalence of initial proximal venous thrombosis in antibody positive patients was a confounding factor. Whether preexisting anticardiolipin antibodies were the cause, or whether thrombi with a larger anatomical extent are more prone to induce anticardiolipin antibodies, cannot be answered by our study.

The increased mortality in patients with anticardiolipin antibodies has not been demonstrated in previous studies. The excess mortality in the group with elevated anticardiolipin antibodies is not explained by any difference in the occurrence of malignant disease. It is probable that a slightly increased incidence of fatal pulmonary embolism as well as myocardial infarction is the main contributor to the higher mortality in these patients. The prevalence of anticardiolipin antibodies has been reported to be high in young patients with myocardial infarction and associated with recurrent cardiovascular events (18). The risk of fatal thromboembolic stroke in our sample seemed, on the other hand, to be similar in patients with and without anticardiolipin antibodies. In a recent study that screened

Table 3. Causes of Death in Patients Tested for Anticardiolipin Antibodies

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Antibody Positive (n = 150)</th>
<th>Antibody Negative (n = 871)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary embolism, n (%)</td>
<td>3 (2)</td>
<td>3 (0.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mesenteric vein thrombosis, n (%)</td>
<td>1 (1)</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Myocardial infarction, n (%)</td>
<td>5 (3)</td>
<td>11 (1.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Thromboembolic stroke, n (%)</td>
<td>1 (1)</td>
<td>5 (0.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Malignancy, n (%)</td>
<td>5 (3)</td>
<td>17 (2.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Congestive heart failure, n (%)</td>
<td>0</td>
<td>5 (0.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Other cardiac causes, n (%)</td>
<td>1 (1)</td>
<td>7 (0.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Intracranial haemorrhage, n (%)</td>
<td>0</td>
<td>4 (0.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>Chronic respiratory disease, n (%)</td>
<td>0</td>
<td>3 (0.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Other causes, n (%)†</td>
<td>0</td>
<td>9 (1.0)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Fisher’s exact test, two-tailed. Difference is not significant unless otherwise indicated.
† Vascular (2), hemorrhage (2), pneumonia (2), alcohol abuse, AIDS, rheumatoid arthritis.
and not causal. Anticardiolipin antibodies are only an epiphenomenon.

Our comparison of death rates with those expected, based on Swedish national statistics, demonstrate that the patients in the antibody negative group had perhaps lower, and certainly not higher, mortality from thromboembolic causes than expected. Perhaps oral anticoagulant therapy was contributory. On the contrary, patients in the antibody positive group seemed to have a higher mortality than expected from these causes, despite oral anticoagulant therapy.

Our results show that in patients with a first event of venous thromboembolism, treated for 6 months with oral anticoagulants, the risk of recurrence during the following 42 months is higher in those with elevated titers of anticardiolipin antibodies. This is in accordance with previous observations from retrospective studies of patients with primary antiphospholipid syndrome and patients with systemic lupus erythematosus (6,8,9) or of lupus patients with antiphospholipid antibodies (5,7). One small study found no difference in the risk of recurrence (13).

Our results also confirm reports that the predictive value of the anticardiolipin antibody test increases with the antibody level (3,20). However, already at low titers that do not fulfill the criteria of primary antiphospholipid syndrome (21), we observed an increased risk of recurrence. As also described previously (9,10), in patients with anticardiolipin antibodies, the risk of recurrence is markedly increased during the first 6 months after discontinuation of oral anticoagulation, suggesting a rebound phenomenon.

It has been claimed that the clinical profile is similar in patients with primary antiphospholipid syndrome and those combined with systemic lupus erythematosus (8,22). Furthermore, several studies on combinations of these patient groups concluded that oral anticoagulation of high intensity is needed to provide protection against recurrences (4,6,8,9). This does not seem to be true for patients with anticardiolipin antibodies and venous thromboembolism. We did not observe a single recurrence in patients who continued with oral anticoagulant therapy, targeted at an intensity corresponding to an INR 2.0 to 2.85. Our observations have been confirmed by other studies of patients with venous thromboembolism and antiphospholipid syndrome (11,12).

We did not find any correlation between the persistence of the antibodies and recurrent events. Many of the recurrences occurred after the transient antibodies had disappeared. Both of these observations suggest that the anticardiolipin antibodies are only an epiphenomenon and not causal.

Our studies do not answer the question of when oral anticoagulation can be discontinued in these patients. One possibility is that 1 year of anticoagulation targeted at an INR of 2 to 2.85, followed by a regimen of lower intensity indefinitely, is a solution for patients with venous thromboembolism and anticardiolipin antibodies, but this will have to be addressed in future studies.

ACKNOWLEDGMENTS
Grants were obtained from Swedish Heart Lung Foundation, the Swedish Society of Medicine, the funds of the Karolinska Institute, Skandia, Trygg-Hansa, Triolab, Nycomed, and Stago.

REFERENCES


**APPENDIX**

The DURAC Trial Study Group consists of the following investigators (the principal investigator of each hospital is in italics). The institutions are, if not otherwise stated, departments of internal medicine.