Acquired Activated Protein C Resistance Caused by Lupus Anticoagulants

Adam J. Saenz, MD, MS, Nicholas V. Johnson, and Elizabeth M. Van Cott, MD

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Abstract

Lupus anticoagulants (LA) can cause acquired activated protein C resistance (APC-R), but the clinical significance is unclear. To investigate thrombosis and acquired APC-R in patients with LA, we enrolled all 132 patients undergoing hypercoagulability testing with positive LA results and in whom APC-R (with factor V–deficient plasma) was performed during a 2.5-year period. Among 121 patients without factor V Leiden, 24.0% had acquired APC-R; retrospective and prospective (mean follow-up, 2.0 years) thrombotic events were analyzed.

The distribution of venous vs arterial thrombosis was different for APC-R vs no APC-R (P = .0064). The majority (19/29 [66%]) with acquired APC-R experienced venous thrombosis, whereas a minority experienced arterial thrombosis (9/29 [31%]; P = .017). The opposite pattern occurred among patients without APC-R (arterial thrombi more common than venous thrombi).

After excluding thrombotic events more than 5 years from a positive LA test, venous thrombosis occurred in 62% with (18/29) vs 32% without (29/92) APC-R (P = .0045); and arterial thrombosis in 28% with (8/29) vs 51% without (47/92) APC-R (P = .033).

Patients with acquired APC-R due to LA had more venous thrombosis than did patients with LA without APC-R and experienced venous more often than arterial thrombosis.

Lupus anticoagulants (LA) are a heterogeneous group of antibodies that are directed against various proteins bound to phospholipid such as prothrombin, β2-glycoprotein I, and annexin V.1 LA are a risk factor for venous and/or arterial thrombosis or recurrent pregnancy loss.2 LA can occur without any underlying illness, or they can occur in association with conditions such as systemic lupus erythematosus.

LA can cause activated protein C resistance (APC-R) in vitro in the absence of factor V Leiden, but the clinical significance is unclear. Hereditary APC-R is caused by factor V Leiden, which is associated with a 3- to 7-fold increased risk for venous thrombosis.3,4 Therefore, we hypothesized that acquired APC-R in patients with LA could be associated with venous thrombosis. In contrast, there is no consistent association between arterial thrombosis and factor V Leiden,5,6 and, therefore, we hypothesized that patients with acquired APC-R due to LA would not have an increased risk for arterial thrombosis. An alternative possibility is that acquired APC-R with LA is an artifact due to LA interference in the assay, in which case there would be no particular association between acquired APC-R and thrombosis in patients with LA. We investigated the relationship of venous thrombosis, arterial thrombosis, and acquired APC-R in patients with LA, and we found evidence that acquired APC-R promotes venous but not arterial thrombosis in patients with LA.

Materials and Methods

We prospectively enrolled all patients undergoing hypercoagulability testing at Massachusetts General Hospital, Boston, in which LA testing was positive and APC-R testing...
was performed during a 2.5-year period. All specimens demonstrated a prolonged LA screen with the partial thromboplastin time PTT-LA assay (Diagnostica Stago, Asnieres, France) performed on an MDAII coagulation analyzer (bioMerieux, Durham NC; now Trinity Biotech, Bray, Ireland) and a positive confirmatory STACLOT-LA hexagonal phase LA assay performed on a START-4 coagulation analyzer (Diagnostica Stago). Note that the PTT-LA is designed to be a screening assay that requires confirmatory testing with the hexagonal phase or other confirmatory assay, and the 2 tests function together as a single partial thromboplastin time (PTT)-based LA test system. In accordance with International Society on Thrombosis and Haemostasis criteria, specimens with positive results for an LA demonstrated prolongation of a clotting time with dilute phospholipid, testing included mixing with normal plasma, clotting times shortened when excess phospholipid was added, and it was determined that the positive results were not due to other inhibitors that could possibly cause false-positive results.

The Coatest APC Resistance V assay (Chromogenix/DiaPharma, West Chester, OH) was used on a STA-R analyzer (Diagnostica Stago) to test for APC-R. This APC-R assay is an activated PTT-based assay that involves a 1:5 dilution with factor V–deficient plasma, and the results are expressed as a ratio. DNA analysis for factor V Leiden and prothrombin G20210A was performed using the Invader assay (Third Wave Technologies, Madison, WI).

The cutoff for APC-R was determined in our laboratory and was confirmed with DNA testing for factor V Leiden. The cutoff was verified for every lot of reagent, and the cutoff was found to be 2.0 throughout the study.

We defined an APC-R ratio of more than 2 as negative and an APC-R ratio less than 2 as positive for acquired APC-R and stratified the 2 groups according to presence or absence of venous or arterial thrombosis. After study enrollment, patients were followed up prospectively, and any additional thrombotic events documented in the medical record were included in the analysis. Statistical analysis was performed on these subgroups using a Fisher exact test and calculation of an odds ratio with the 95% confidence interval (CI). In addition, calculation of a 2 \times 4 Fisher P value was conducted using a computer program designed by one of us (N.V.J.). Logistic regression was used to assess APC-R as a continuous variable vs venous and arterial thrombosis.

In accordance with new international consensus guidelines for antiphospholipid antibody syndrome, we analyzed the data after excluding thrombotic events that occurred more than 5 years before a positive LA test. For comparison, data including these older thrombotic events were also analyzed. Institutional review board approval was obtained for the study.

Venous thrombotic events (deep venous thromboses and/or pulmonary emboli) were diagnosed by objective radiographic tests, specifically lower or upper extremity ultrasound and/or extremity/pelvis/chest computed tomography (CT) scan. Arterial thrombotic events were confirmed by the following objective tests: CT and/or magnetic resonance imaging for ischemic stroke and serial cardiac markers with electrocardiogram for myocardial infarction. Of the myocardial infarctions, 5 in the no APC-R group occurred at outside hospitals, and cardiac enzyme/electrocardiogram results were not available, but 4 of the 5 patients also had an objectively documented second type of arterial thrombotic event, and the fifth case was excluded in the analysis that excluded events that were more than 5 years old. Transient ischemic attack was diagnosed by a neurologist’s assessment. A small number of other types of arterial thrombotic events were diagnosed by magnetic resonance imaging, CT, thrombectomy, or histologic analysis by a pathologist.

Results

We identified 132 patients who met the criteria for study entry during the 2.5-year (30-month) study enrollment period, with a mean age of 52.6 years (range, 17-89 years), 76 of whom were female (57.6%). The DNA assay for factor V Leiden was normal in 121 patients, and heterozygous factor V Leiden was identified in the remaining 11 patients. Among the 121 patients without factor V Leiden, 29 (24.0%) had acquired APC-R (APC-R ratio <2). The mean APC-R ratio among patients with LA with acquired APC-R was 1.71, which was similar to the mean of the 11 patients with LA with heterozygous factor V Leiden (1.74). The mean APC-R ratio among patients with LA without APC-R (normal APC-R ratios) was 2.47.

A total of 89 of 121 patients experienced a thrombotic event at or before study enrollment. In addition, the 121 patients were followed up prospectively for a mean of 2.0 years (range, 222-1,479 days among surviving patients and 5-201 days among the 7 patients who died), during which time 21 patients experienced 27 thrombotic events, and 2 of these patients had no history of thrombosis at baseline.

A recent consensus statement recommended that thrombotic events more than 5 years from a positive antiphospholipid antibody test or superficial thrombophlebitis should not qualify as clinical criteria for antiphospholipid antibody syndrome. Therefore, the data were analyzed before and after excluding the 12 venous or arterial thrombotic events that occurred more than 5 years from a positive antiphospholipid antibody test (2 with APC-R and 10 with normal APC-R). Superficial venous thrombophlebitis
occurred in 10 patients (1 with APC-R and 9 with normal APC-R) but did not affect the results because all 10 patients also had deep venous thrombosis.

The majority of patients with acquired APC-R (19/29 [66%]) experienced a venous thrombotic event, whereas only a minority experienced an arterial thrombotic event (9/29 [31%]), a difference that was statistically significant ($P = .017$; odds ratio for venous vs arterial thrombosis, 4.2; 95% CI, 1.4-12.7). After excluding thrombotic events that occurred more than 5 years from a positive LA test, the results remained significant (18/29 vs 8/29; $P = .017$; odds ratio, 4.3; 95% CI, 1.4-13.0). In contrast, the opposite pattern was seen among the 92 patients with normal APC-R (no APC-R) in that arterial thrombi were more common than venous thrombi (significant only after excluding thrombotic events >5 years from a positive LA test: 29/92 with venous vs 47/92 with arterial thrombosis; $P = .011$; odds ratio for venous vs arterial thrombosis, 0.44; 95% CI, 0.24-0.80).

A comparison of the APC-R vs no APC-R groups showed that 19 (66%) of 29 patients with APC-R had venous thrombosis vs 37 (40%) of 92 patients without APC-R ($P = .020$; odds ratio, 2.8; 95% CI, 1.2-6.8). After excluding the events that occurred more than 5 years from a positive LA test, there remained significantly more venous events in the APC-R group than in the normal APC-R group: 18/29 vs 8/29; $P = .0045$; odds ratio, 3.6; 95% CI, 1.5-8.5). The opposite pattern was seen for arterial events: only 9 (31%) of 29 patients with APC-R vs 29 (32%) of 92 without APC-R ($P = .008$) and an inverse relationship of APC-R as a continuous variable with arterial thrombosis ($P = .041$).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>APC-R (n = 29)</th>
<th>No APC-R (n = 92)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td>18 (62)</td>
<td>29 (32)</td>
<td>.0045</td>
</tr>
<tr>
<td>Arterial</td>
<td>8 (28)</td>
<td>47 (51)</td>
<td>.033</td>
</tr>
</tbody>
</table>

Table 1 shows APC-R vs arterial thrombosis in LA with vs without APC-R.

When looking only at the thrombotic events that occurred during prospective follow-up, the same pattern of results was observed. Arterial thrombosis occurred in 19% of patients without (17/92) vs only 7% in patients with APC-R (2/29). Venous thrombosis occurred in 2% of patients without (2/92) vs 7% of patients with (2/29) APC-R. When including the 3 additional thrombotic events that occurred after the study period closed, the difference increased further: 20% without (18/92) vs 7% with (2/29) APC-R had arterial thrombosis, and 3.3% without (3/92) vs 10% with (3/29) APC-R had venous thrombosis during prospective follow-up.

**APC-R as a Continuous Variable**

Logistic regression found a significant association between APC-R as a continuous variable with venous thrombosis ($P = .008$) and an inverse relationship of APC-R as a continuous variable with arterial thrombosis ($P = .041$). Figure 1 shows APC-R across the spectrum of values vs venous thrombosis. For patients with APC-R, there seems to be more venous thrombosis with lower APC-R ratios (more abnormal results).

**Other Thrombophilia Tests**

All but 3 patients were also tested for protein C, protein S, or antithrombin deficiency, which showed no difference between the APC-R and no APC-R groups. Of 92 patients with normal APC-R, 3 had possible hereditary protein C deficiency (an acquired cause could not be identified); 2 of the patients had venous thrombosis and 1 had no thrombosis. None of the patients with APC-R had protein C deficiency, and no patients (APC-R or no APC-R) had hereditary antithrombin deficiency. Only 1 patient had possible hereditary protein S deficiency (an acquired cause could not be identified); this patient had APC-R and arterial but no venous thrombosis. Thus, excluding these few patients with possible hereditary deficiencies would only further strengthen the association between acquired APC-R and venous thrombosis.

Prothrombin G20210A testing, performed at the discretion of the patients’ physicians, also showed no significant difference between the APC-R and no APC-R groups ($P = .32$; 2 of 21 patients with APC-R were heterozygous, one had no
thrombosis and the other had venous and arterial thrombosis; 2 of 53 patients without APC-R were heterozygous, one had no thrombosis and the other had venous thrombosis; no homozygous patients were identified).

Relation of PTT-LA Prolongation to APC-R

The presence of acquired APC-R seemed related to the degree that the LA can prolong the PTT-LA (the PTT-based LA screening assay), but this did not account for the association with thrombosis because the PTT-LA prolongation was unrelated to venous thrombosis or arterial thrombosis. Specifically, the mean PTT-LA was 72.1 seconds in the APC-R group vs 55.3 seconds in the normal APC-R group \((P = .0046)\), whereas the mean PTT-LA was actually shorter among patients with venous thrombosis (55.9 seconds) vs patients without venous thrombosis (60.1 seconds; \(P = .40\)). Similarly, the mean PTT-LA was virtually identical among patients with arterial thrombosis (58.9 seconds) vs patients without arterial thrombosis (58.1 seconds; \(P = .87\)).

Persistence of APC-R

To assess the persistence (or transience) of acquired APC-R, medical records were reviewed to obtain repeated APC-R test results, past or present. In 8 patients with acquired APC-R, repeated testing was done a median of 1.8 months apart (range, 3 days to 9 years), and in all 8 cases, APC-R was still present. In 23 patients with normal APC-R, repeated testing was done a median of 5.0 months apart (range, 1 day to 13 years), and in all cases, APC-R was still normal. Outside of this study, we have previously observed that LA-induced acquired APC-R returns to normal when or if the LA disappears.\(^9\)

Discussion

To our knowledge, this is the first study to examine the incidence of venous vs arterial thrombosis in patients with LA with vs without APC-R using the commonly used “modified” APC-R assay (ie, in factor V–deficient plasma). Our study raises the possibility that patients with acquired APC-R due to LA have a greater risk for venous thrombosis than they do for arterial thrombosis. The results also raise the possibility that patients with acquired APC-R due to LA have an increased risk for venous thrombosis compared with patients with LA with normal APC-R. These findings biologically mimic a true factor V Leiden. These results suggest that the acquired APC-R due to LA is not just an in vitro artifact, but rather, that it may have in vivo effects on venous thrombotic risk. Furthermore, patients with LA with acquired APC-R did not have an increased risk for arterial thrombosis compared with patients with LA without APC-R and, in fact, had fewer arterial events, which further mimics factor V Leiden because there is no clear association between factor V Leiden and arterial events.\(^5,6\) This finding might help explain the mechanism of thrombosis in the subgroup of patients with LA who have APC-R due to LA. In addition, if this finding is substantiated in further study, the results suggest that testing for APC-R in patients with LA may help predict which type of thrombosis (venous or arterial) is their largest risk.

The modified APC-R assay used in the present study is widely used in clinical laboratories and is more commonly used than the original APC-R assay. In the College of American Pathologists 2007 CED-B proficiency test, 107 laboratories reported using the modified assay (ie, a PTT ratio with factor V–deficient plasma), whereas 16 laboratories reported using the original assay (ie, a PTT ratio without factor V–deficient plasma). Because clinical laboratories commonly use the modified assay, the present study was conducted to determine if additional information regarding thrombosis risk can be obtained from the modified APC-R assay in patients with LA. Furthermore, the specific assay used in the present study (Coatest APC Resistance V) is used by the majority of clinical laboratories, according to
a December 2009 proficiency testing report by the ECAT Foundation (formerly called European Concerted Action on Thrombosis), which includes laboratories from a variety of continents including North America.

The modified APC-R assay is very accurate in detecting factor V Leiden. In our clinical laboratory at Massachusetts General Hospital, the modified APC-R assay used in this study was implemented in the mid-1990s, and between 2001-2009, it was performed in 30,477 cases. Factor V Leiden DNA testing is performed on all specimens with APC-R values of 2.1 or less or specimens with LA or argatroban (n = 3,051 between 2001-2009), and since testing began in the mid-1990s, all specimens with values less than 2.0 have tested positive for factor V Leiden, except for an occasional LA specimen due to acquired APC-R. These results highlight the exceptional specificity of this particular modified assay for factor V Leiden and indicate that LA are virtually the only entity that can cause an abnormal result that is not factor V Leiden with this assay. (Other reported causes, such as factor V Cambridge, are apparently very rare; in contrast, other types of APC-R assays, such as the original assay, can be abnormal in other conditions besides factor V Leiden or LA.) Our clinical laboratory also "reflexes" LA specimens for DNA testing even when the APC-R value is normal (>2.1), and all LA cases with normal APC-R have tested negative for factor V Leiden since this assay was implemented in the mid-1990s. Therefore, it seems that LA do not cause false-normal APC-R results, and it may not be necessary to continue to do factor V Leiden DNA testing in patients with LA who have normal APC-R values with this particular assay.

In contrast, the original APC-R assay is affected by numerous interferences, including elevated factor VIII (eg, during acute phase reactions or pregnancy), oral contraceptives, heparin, factor deficiencies, warfarin and other vitamin K antagonists, vitamin K deficiency, and liver dysfunction. For example, a patient with venous thrombosis could have an elevated factor VIII level as a result of an acute phase reaction in response to the thrombotic event, which would cause APC-R with the original assay. Thus, an apparent reaction in response to the thrombotic event, which would elevate factor VIII level as a result of an acute phase reaction, might be due to an acute phase reaction to the thrombosis. In contrast, the modified APC-R assay (used in the present study) is not interfered with by any of these conditions.

An alternative APC-R assay called Pefakit (Centerchem, Norwalk, CT) is not affected by lupus anticoagulants according to the manufacturer. Future study could include this assay to confirm whether it can detect an acquired APC-R due to LA.

In our study, among patients without APC-R, the mean baseline PTT-based clotting time (denominator of the APC-R ratio) was 42.1 seconds, and the mean clotting time after adding APC was 103.7 seconds (numerator of the APC-R ratio). Among patients with APC-R, the mean baseline clotting time was 64.7 seconds, and the mean clotting time after adding APC was 108.6 seconds. LA-induced prolongation of the baseline clotting time does not explain the reduced APC-R ratio in patients with APC-R because the LA did not prolong their clotting times with added APC to a normal extent (a 2-fold or more prolongation over corresponding baseline would be normal). In general, if an LA can prolong a baseline clotting time, it is even more capable of prolonging an already-prolonged clotting time, such as a clotting time prolonged by APC. In the present study, because the only difference between the 2 clotting times is added APC, the reduced prolongation with added APC raises the possibility of APC-R.

This study raises the possibility that patients with acquired APC-R due to LA have an increased risk for venous thrombosis compared with patients with LA with normal APC-R. The results also raise the possibility that patients with acquired APC-R due to LA have a greater risk for venous thrombosis than they do for arterial thrombosis. These characteristics of acquired APC-R due to LA mimic APC-R due to factor V Leiden, which is associated with an increased risk for venous but not arterial thrombosis. If these findings are substantiated by further study, the results suggest that testing for APC-R in patients with LA might help predict which type of thrombosis (venous or arterial) is their largest risk. Further inquiry is needed in a larger cohort of patients to understand the pathophysiology and therapeutic strategies.

References


