EDITORIAL

Lupus Anticoagulant, Anticardiolipin Antibodies, Fetal Loss, and Systemic Lupus Erythematosus

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LUPUS ANTICOAGULANTS (LA) and anticardiolipin (ACL) antibodies (ACLA) belong to a heterogeneous group of antibodies directed against negatively charged phospholipids. However, more recent studies would suggest that these antibodies are directed to epitopes of other proteins that are complexed with negatively charged phospholipids. Although antiphospholipid antibodies are frequently found in otherwise healthy persons, the presence of these antibodies is closely associated with the occurrence of arterial and venous thromboembolism, thrombocytopenia, fetal loss, and a variety of other conditions in patients with and without systemic lupus erythematosus (SLE). However, the nature of the association between antiphospholipid antibodies and these clinical events, and whether they are directly causative or rather represent a consequence of the clinical condition with no direct pathophysiologic role, is unknown.

ACLA are usually detected by solid-phase immunoassays, whereas the LA is measured as an activity that prolongs lipid-dependent clotting reactions assayed by coagulation techniques. Because both antibodies react with phospholipids and are frequently found in the plasma of the same patients, it has been suggested that they are identical. However, in a number of studies, ACLA can be separated from LA activity, and in many patients the activities are discordant. Therefore, performing assays for both types of antiphospholipid antibodies in a given patient maximizes the likelihood of detecting these activities.

Although testing for ACLA is now well standardized, tests for LA have not been well standardized, and there is no established reliable method for their quantitation. Many tests for the detection of LA activity have been described, but there is little agreement among workers in the field regarding the optimum test for detecting LA activity. However, it is generally agreed that coagulation assays with either dilute or no phospholipid are the most sensitive, whereas correction of the prolonged clotting time by added phospholipid increases specificity. Moreover, all plasma used in testing should be centrifuged at 5 to 15,000g for 10 to 15 minutes or filtered to render them platelet free and avoid activity that can neutralize the LA.

Although it has been recognized that LA and ACLA activity can fluctuate in a given patient over time, similar to other autoantibodies, correlation of clinical events with the presence of these antibodies in patients with or without SLE is very meager. Recently, in an attempt to answer this question, Long et al performed a cross-sectional study of 69 unselected consecutive patients with SLE using five coagulation assays for the LA and an enzyme-linked immunosorbent assay (ELISA) for ACLA. These tests were measured on two separate occasions and then correlated with specific objective criteria for the diagnosis of venous and/or arterial thromboembolic disorders. The patients were considered to be persistently positive for the LA if one or more of the coagulation tests was positive on both occasions, transiently positive if one or more was positive on one occasion and all tests were negative on the other, and negative if all five tests were normal on both occasions. Patients were considered to be persistently positive for ACLA if the IgG and/or IgM assays were positive on both occasions, whereas they were considered to be transiently positive if the test was positive on one occasion and negative on the other. Twenty-four of the 69 patients (34.8%) were persistently LA positive, 7 (10%) were transiently positive, and 38 (55.1%) were persistently negative. Fourteen of the 24 patients who had persistently positive LA tests had different profiles of positivity with respect to the five clotting assays used at different times. Fifteen of the 69 patients (21.7%) were ACLA positive, 14 (24.3%) were transiently positive, and 40 (58%) were persistently negative. Statistically significant associations were shown between prior thromboembolic events and positive ACLA alone and both LA and ACL positivity, whereas there was an insignificant trend between thromboembolism and LA positivity alone. Interestingly, the strength of the association between LA/ACLA positivity was much reduced when transiently positive patients were included as positive. In this study, there was no coagulation test that best correlated with thromboembolic events, and the results of the same LA clotting test and the ELISA for ACLA changed frequently in individual patients.

In this issue of Blood, the same group who performed the above study applied a similar investigative design in an attempt to determine whether an association exists between fetal loss and the presence of antiphospholipid antibodies in patients with SLE. Although several studies have suggested such a relationship in patients with SLE, a recent review of the published studies could not unequivocally establish a definite association. Moreover, in a recent well-controlled prospective cohort study in patients without SLE presenting with an initial episode of fetal loss, no relationship could be established between the presence of antiphospholipid antibodies and fetal loss. In the study published in this issue, 42 consecutive patients with SLE with 122 pregnancies and with a history of at least one pregnancy loss were studied; 11 had a history of one pregnancy loss and 7 had two or more pregnancy losses. A history of pregnancy loss occurred in 24 of the 55 pregnancies in patients with persistent LA positivity compared with...
8 of 67 pregnancies in LA-negative patients ($P < .0001$). However, similar to patients with thromboembolic disease, if transiently positive patients were grouped with persistently positive patients, the significance decreased. Thus, repeat positivity was more strongly associated with pregnancy loss than transient positivity. Moreover, all seven of the women experiencing multiple pregnancy loss showed persistently positive tests.

Similar results were found for ACLA in that 10 of 14 pregnancies in patients who were persistently positive resulted in pregnancy loss compared with 22 of 108 pregnancies in patients who were negative or transiently positive. All five women who were persistently positive had pregnancy loss, compared with 13 of 37 patients who were negative or transiently positive with no pregnancy loss. Similar to the patients with LA positivity, if patients who were transiently positive were grouped with patients who were persistently positive, the significance decreased. Thus, repeat ACLA positivity, similar to LA positivity, was more strongly associated with pregnancy loss than transient positivity. Interestingly, in this group of patients, all who were positive for ACLA were also positive for LA activity. Although the latter pattern of concordance has been noted in previously reported patients, discordant results are common.$^{2,12-14}$

In the 15 patients who were repeatedly positive for LA activity, only 5 had identical patterns of testing, whereas 10 patients had different patterns of positivity. Furthermore, no one test appeared to be superior to another in correlating with pregnancy loss. Interestingly, in the women who were repeatedly positive for LA, there was a greater pregnancy loss (19 of 35) in those who had 6 to 10 positive tests compared with those who had 2 to 5 positive tests (5 of 20). Moreover, the prevalence of repeatedly abnormal individual tests in patients with pregnancy loss was low compared with combining all five test results. Thus, these results would strongly support the hypothesis that a combination of coagulation tests for LA activity plus testing for ACLA on more than one occasion is superior to a single test at one period of time in attempting to correlate the laboratory results with clinical events. The results from this study regarding fetal loss are similar to this group’s results regarding the relationship of antiphospholipid antibodies to thromboembolic disorders. In addition, the lack of consistent results with a single LA and ACLA test suggests a possible explanation for the conflicting results regarding the association of antiphospholipid antibodies with pregnancy loss in previous studies.$^{25}$

Because of the cross-sectional and retrospective design of the study, the temporal relationship between the development of antiphospholipid antibodies, SLE, and fetal wastage could not be determined. Some of the patients had not even been diagnosed as having SLE at the time of their pregnancy. In addition, like many other clinical associations with phospholipid antibodies, whether there is a cause and effect relationship could not be established. As Ginsberg et al point out, only a prospective cohort study design could possibly establish a temporal relationship between the diagnosis of SLE and the development of antiphospholipid antibodies with fetal wastage and/or thromboembolic events.

To answer this important question, patients would have to be studied at the time when the diagnosis of SLE is first established and tested serially over time. If the diagnosis of SLE is made before pregnancy, then it might be possible to establish a temporal and even a cause and effect relationship between the development of antiphospholipid antibodies and a clinical event. In addition, in any future studies it would be important to analyze the data in relation to the number of previous pregnancies and maternal age, which are other risk factors for recurrent fetal loss.

Obviously the results from this study cannot be transposed to patients with antiphospholipid antibodies without SLE. A recent prospective case control study by Infante-Rivard et al$^{26}$ in women without SLE found that women experiencing spontaneous abortion or fetal death were unlikely to have either LA or ACLA more frequently than women in the same period of pregnancy whose pregnancy was normal. However, it is important to point out that this study excluded all patients with previous pregnancy loss, the antiphospholipid antibody studies were performed only once, and only two different tests were used for detection of the LA. It will be of interest to perform serial testing on this cohort of patients as they progress to subsequent pregnancies.

The cause of fetal wastage in these patients is thought to be due to thrombosis of placental vessels with resultant ischemia and infarction. The very high incidence of thrombotic disorders in these patients lends further support to this hypothesis. Despite a great deal of studies and a variety of hypotheses, there is no consensus on the specific pathophysiologic mechanism involved in the possible causation of thrombosis.$^{2}$ Therefore, prospective and serial studies may well help to shed some light on this very important issue.

Although it is well known that autoantibody levels can fluctuate spontaneously, the reasons for such variations in these patients in a relatively short period of time are not readily apparent. Although steroid and other immunosuppressive treatment could possibly have altered the results, there were no significant differences in the proportion of patients on corticosteroids or other immunosuppressive therapy among the different patterns of test results. However, it is important to emphasize that Ginsberg et al considered as abnormal only those LA test results that were two standard deviations above normal and ACL antibody levels that were four standard deviations above normal. Thus, patients whose test results fell in the gray zone were considered to test negative even though the patient possibly had an abnormal test result previously. However, even considering this as a possibility, it does not alter Ginsberg et al’s conclusion that a combination of the tests for LA activity that they used was superior to a single test, and repeating the same tests on another occasion increased the strength of the clinical association. It should be noted that the relatively small number of patients with LA positivity precluded any conclusion that one of the five tests used was superior to the others. Although there is neither a gold standard for LA testing nor a standardized method for quantitating LA, recent comparative studies support the idea that the kaolin clotting time is more sensitive than other methodology and, in addition, can be used to semi-
quantitate LA activity.\textsuperscript{18-20} It would be of great interest if the kaolin clotting time and the quantitation of LA activity could be included in future serial studies in such patients. The results of the latter would be of particular interest because it has been suggested that fetal loss and thromboembolic events correlate better with the presence of high-titer ACLA.\textsuperscript{2,5,10} Of the five women in the study who repeatedly tested positive for ACLA, four had persistently high titers and one had a high titer on one occasion. Four of these five patients had multiple pregnancy losses. Unfortunately, the small number of patients who were positive for ACLA and the lack of patients with an intermediate titer of antibodies precluded any conclusion regarding the relationship of the titer of ACLA to fetal loss.

In addition to test sensitivity for LA in the causality of differing test results, variation in the level of antibody and/or heterogeneity of the antibodies being detected could also contribute to the variability in test positivity. Variability in the level of antibody could be due to modulation of the patient’s immune response and/or variability in the amount of antigen present. It has recently been shown that the interaction of ACLA with anionic phospholipid requires the presence of a plasma protein, \(\beta_2\)-glycoprotein I (\(\beta_2\)-GPI).\textsuperscript{6,8} In a recent report, levels of \(\beta_2\)-GPI were measured in 43 patients who were antiphospholipid antibody positive.\textsuperscript{27} Interestingly, ACLA-positive patients who were negative for LA activity had normal levels of \(\beta_2\)-GPI, whereas LA-positive patients had significantly increased levels of \(\beta_2\)-GPI regardless of the ACLA levels. There is no ready explanation for these data. However, it strongly suggests that \(\beta_2\)-GPI levels do vary in patients with the LA and might in some way be related to the variation in test results in patients with the LA. It would obviously be of interest to measure \(\beta_2\)-GPI levels serially in those patients and correlate those results with the results of LA and ACLA levels.

Whether \(\beta_2\)-GPI is the same cofactor that in normal plasma potentiates the LA in clotting assays has not been clearly established. In fact, in a recent study by Bevers et al,\textsuperscript{3,9} it was found that when LA activity was separated from ACLA, the prolongation of the clotting times in the presence of isolated LA activity required the presence of both anionic phospholipid and human prothrombin. Thus, the Bevers et al hypothesized that the LA was an antibody directed to a human prothrombin-phospholipid complex analogous to ACLA being directed to a \(\beta_2\)-GPI–phospholipid complex. This would corroborate previous studies suggesting that the antibodies responsible for LA activity exhibit polyreactivity for both prothrombin and phospholipid.\textsuperscript{28,29} In contrast, in recent studies by Galli et al,\textsuperscript{3,7} when \(\beta_2\)-GPI was added to normal plasma in concentrations comparable to the elevated levels found in LA plasma, it resulted in significant prolongation of the partial thromboplastin time and the kaolin clotting time similar to the original cofactor effect described by Loeliger.\textsuperscript{30} Thus, this issue remains controversial, and more studies are needed to identify the activity in normal plasma that causes further prolongation of the clotting time when added to plasma that contains the LA.

Identification of a definite association of antiphospholipid antibodies with fetal loss has important implications in that different therapeutic regimens in uncontrolled trials have resulted in what appears to be a significant decrease in fetal loss.\textsuperscript{27,31,32} Although some studies with high-dose corticosteroids and low-dose aspirin have resulted in a decrease in fetal loss, long-term high-dose steroids during pregnancy are associated with significant side-effects, including preeclampsia, infection, gestational diabetes, cushingoid features, and osteoporosis. Moreover, not all reports using this regimen or aspirin alone have shown efficacy.\textsuperscript{34} In a recent and important report by Rosove et al,\textsuperscript{3,5} the use of adjusted full-dose subcutaneous heparin showed a significant decrease in fetal loss. In this report, 14 women (five with SLE) with 28 previous fetal losses were treated with adjusted full-dose subcutaneous heparin. Of 15 pregnancies, 14 resulted in live births. None of the patients were treated with aspirin and only two patients received a short course of corticosteroids for concomitant autoimmune disease. In addition, in a few anecdotal case reports,\textsuperscript{36,37} intravenous IgG also resulted in fetal salvage.

Finally, what should a clinician do at this time with this very interesting and provocative data? It is very clear that patients at risk (any patient with SLE and those with unexplained fetal loss or intrauterine growth retardation) should be tested for both the LA and ACLA. If both tests are negative, then repeat testing need not be performed unless a clinical event occurs that is known to be associated with antiphospholipid antibodies. If one or both tests are positive, then the patient should undergo repeat testing 2 to 4 months later. Although this study implies that several tests should be used for the LA, it may be sufficient and more practical at this time to perform a kaolin clotting time\textsuperscript{30} or a dilute phospholipid partial thromboplastin time.\textsuperscript{27} If a patient is persistently positive for either LA or ACLA and has never been pregnant or has had no history of pregnancy loss, then the patient should be counseled regarding the risk of possible fetal loss and closely observed whenever she becomes pregnant. In contrast, if the patient has a history of recurrent fetal loss, then therapeutic intervention with heparin is probably indicated. Whether a patient with SLE with a single first trimester spontaneous abortion should receive treatment is not completely clear at this time, but more data are needed. Hopefully, future studies of these antibodies and careful clinical observations will be successful in establishing a temporal relationship with clinical events and determine whether these antibodies are a direct cause of fetal loss or thromboembolism.

REFERENCES


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